𝔜ale 𝔅icentennial 𝔓ublications

STUDIES IN PHYSIOLOGICAL CHEMISTRY

Yale Bicentennial Publications

With the approval of the President and Fellows of Yale University, a series of volumes has been prepared by a number of the Professors and Instructors, to be issued in connection with the Bicentennial Anniversary, as a partial indication of the character of the studies in which the University teachers are engaged.

This series of volumes is respectfully dedicated to

The Graduates of the University

STUDIES

IN

PHYSIOLOGICAL CHEMISTRY

*BEING REPRINTS OF THE MORE IMPORTANT STUDIES
ISSUED FROM THE LABORATORY OF
PHYSIOLOGICAL CHEMISTRY*

SHEFFIELD SCIENTIFIC SCHOOL

OF

YALE UNIVERSITY

DURING THE YEARS 1897–1900

EDITED BY

R. H. CHITTENDEN, PH.D.

*Professor of Physiological Chemistry and Director of the Sheffield
Scientific School*

TABLE OF CONTENTS.

BIBLIOGRAPHY

1875   On Glycogen and Glycocoll in the Muscular Tissue of *Pecten irradians*. By R. H. CHITTENDEN. American Journal of Science and Arts (3), vol. X, pp. 26-32.

On some interesting Equine Calculi. By R. H. CHITTENDEN. American Journal of Science and Arts (3), vol. X, pp. 195-196.

1876   On the Oxidation Product of Glycogen with Bromine, Silver Oxide, and Water. By R. H. CHITTENDEN. American Journal of Science and Arts (3), vol. XI, pp. 395-401.

1877   On the Chemical Composition of the Flesh of Hippoglossus Americanus. By R. H. CHITTENDEN. American Journal of Science and Arts (3), vol. XIII, pp. 123-124.

1878   On Schweitzer's New Acid Ammonium Sulphates. By S. W. JOHNSON and R. H. CHITTENDEN. American Journal of Science and Arts (3), vol. XV, pp. 131-134.

1880   On the Detection and Determination of Arsenic in Organic Matter. By R. H. CHITTENDEN and H. H. DONALDSON. American Chemical Journal, vol. II, pp. 235-244.

On the Distribution of Arsenic in the Human Body in a Case of Arsenical Poisoning. By S. W. JOHNSON and R. H. CHITTENDEN. American Chemical Journal, vol. II, pp. 332-337.

1881   On the Diastatic Action of Saliva. By R. H. CHITTENDEN and W. L. GRISWOLD. American Chemical Journal, vol. III, pp. 305-316.

1882   On Arsenical Bismuth-Subnitrate. By R. H. CHITTENDEN and S. W. LAMBERT. American Chemical Journal, vol. III, pp. 396-402.

Influence of Peptones and certain Inorganic Salts on the Diastatic Action of Saliva. By R. H. CHITTENDEN and J. S. ELY. The Journal of Physiology, vol. III, pp. 327-342.

1883 On the Alkalinity and Diastatic Action of Human Saliva. By R. H. CHITTENDEN and J. S. ELY. American Chemical Journal, vol. IV, pp. 329–333.

On the Distribution of Arsenic in a Human Body. By R. H. CHITTENDEN. American Chemical Journal, vol. V, pp. 8–14.

Ueber die nächsten Spaltungsproducte der Eiweisskörper. By W. KÜHNE and R. H. CHITTENDEN. Zeitschrift für Biologie, Band XIX, pp. 159–208.

1884 Ueber Albumosen. By W. KÜHNE and R. H. CHITTENDEN. Zeitschrift für Biologie, Band XX, pp. 11–51.

On some New Forms of Albumose. By W. KÜHNE and R. H. CHITTENDEN. American Chemical Journal, vol. VI, pp. 31–50 and pp. 101–120.

On Palmitic Acid and the Palmitins. By R. H. CHITTENDEN and H. E. SMITH. American Chemical Journal, vol. VI, pp. 217–233.

On the Relative Digestibility of Fish Flesh in Gastric Juice. By R. H. CHITTENDEN and GEORGE W. CUMMINS. American Chemical Journal, vol. VI, pp. 318–332.

1885 The Diastatic Action of Saliva, as modified by various conditions, studied quantitatively. By R. H. CHITTENDEN and H. E. SMITH. Transactions Connecticut Academy of Arts and Sciences, vol. VI, pp. 343–375. Studies from Laboratory Physiological Chemistry, Yale University, vol. I, pp. 1–34.

The Amylolytic Action of Diastase of Malt, as modified by various conditions, studied quantitatively. By R. H. CHITTENDEN and GEORGE W. CUMMINS. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 44–59. Studies from Laboratory Physiological Chemistry, Yale University, vol. I, pp. 36–51.

Influence of certain Therapeutic and Toxic Agents on the Amylolytic Action of Saliva. By R. H. CHITTENDEN and H. M. PAINTER. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 60–83. Studies from Laboratory Physiological Chemistry, Yale University, vol. I, pp. 52–75.

Influence of Bile, Bile Salts, and Bile Acids on Amylolytic and Proteolytic Action. By R. H. CHITTENDEN and GEORGE W. CUMMINS. American Chemical Journal, Vol. VII, pp. 36–52. Studies from Laboratory Physiological Chemistry, Yale University, vol. I, pp. 126–140.

Influence of various Inorganic and Alkaloid Salts on the Proteolytic Action of Pepsin-Hydrochloric Acid. By R. H. CHITTENDEN and S. E. ALLEN. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 84–107. Studies from Laboratory Physiological Chemistry, Yale University, vol. I, pp. 76–99.

Absorption of Arsenic by the Brain. By R. H. CHITTENDEN and H. E. SMITH. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 149–152.

Influence of various Therapeutic and Toxic Substances on the Proteolytic Action of the Pancreatic Ferment. By R. H. CHITTENDEN and GEORGE W. CUMMINS. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 108–124. Studies from Laboratory Physiological Chemistry, Yale University, vol. I, pp. 100–116.

Influence of Temperature on the Relative Amylolytic Action of Saliva and the Diastase of Malt. By R. H. CHITTENDEN and W. E. MARTIN. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 125–133. Studies from Laboratory Physiological Chemistry, Yale University, vol. I, pp. 117–125.

Influence of Potassium and Ammonium Bromides on Metabolism. By R. H. CHITTENDEN and W. L. CULBERT. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 153–165. Studies from Laboratory Physiological Chemistry, Yale University, vol. I, pp. 145–157.

Influence of Cinchonidine Sulphate on Metabolism. By R. H. CHITTENDEN and HENRY H. WHITEHOUSE. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 166–178. Studies from Laboratory Physiological Chemistry, Yale University, vol. I, pp. 158–170.

The Post-Mortem Formation of Sugar in the Liver in the presence of Peptones. By R. H. CHITTENDEN and ALEXANDER LAMBERT. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 179–206. Studies from Laboratory Physiological Chemistry, Yale University, vol. I, pp. 171–198.

1886 Globulin und Globulosen. By W. KÜHNE and R. H. CHITTENDEN. Zeitschrift für Biologie, Band XXII, pp. 409–422. Studies from Laboratory Physiological Chemistry, Yale University, vol. II, pp. 1–13.

Ueber die Peptone. By W. KÜHNE and R. H. CHITTENDEN. Zeitschrift für Biologie, Band XXII, pp. 423–458. Studies from

Laboratory Physiological Chemistry, Yale University, vol. II, pp. 14–45.

On the Dehydration of Glucose in the Stomach and Intestines. By R. H. CHITTENDEN. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 252–259. Studies from Laboratory Physiological Chemistry, Yale University, vol. II, pp. 46–53.

Influence of Uranium Salts on the Amylolytic Action of Saliva and the Proteolytic Action of Pepsin and Trypsin. By R. H. CHITTENDEN and M. T. HUTCHINSON. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 261–273. Studies from Laboratory Physiological Chemistry, Yale University, vol. II, pp. 55–67.

The Relative Distribution of Antimony in the Organs and Tissues of the Body under Varying Conditions. By R. H. CHITTENDEN and JOSEPH A. BLAKE. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 274–292. Studies from Laboratory Physiological Chemistry, Yale University, vol. II, pp. 68–86.

Influence of Antimonious Oxide on Metabolism. By R. H. CHITTENDEN and JOSEPH A. BLAKE. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 293–300. Studies from Laboratory Physiological Chemistry, Yale University, vol. II, pp. 87–94.

On some Metallic Compounds of Albumin and Myosin. By R. H. CHITTENDEN and HENRY H. WHITEHOUSE. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 301–331. Studies from Laboratory Physiological Chemistry, Yale University, vol. II, pp. 95–125.

Egg-Albumin and Albumoses. By R. H. CHITTENDEN and PERCY R. BOLTON. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 332–361. Studies from Laboratory Physiological Chemistry, Yale University, vol. II, pp. 126–155.

Casein and its Primary Cleavage Products. By R. H. CHITTENDEN and H. M. PAINTER. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 363–405. Studies from Laboratory Physiological Chemistry, Yale University, vol. II, pp. 156–199.

1887 Influence of some Organic and Inorganic Substances on Gas Metabolism. By R. H. CHITTENDEN and G. W. CUMMINS. Transactions Connecticut Academy of Arts and Sciences, vol. VII, pp. 406–442, 1 pl. Studies from Laboratory Physiological Chemistry, Yale University, vol. II, pp. 200–236.

1888 Some Experiments on the Physiological Action of Uranium Salts. By R. H. CHITTENDEN and ALEXANDER LAMBERT. Transactions Connecticut Academy of Arts and Sciences, vol. VIII, pp. 1–18. Studies from Laboratory Physiological Chemistry, Yale University, vol. III, pp. 1–18.

Elastin and the Elastose Bodies. By R. H. CHITTENDEN and HORACE S. HART. Transactions Connecticut Academy of Arts and Sciences, vol. VIII, pp. 19–38. Studies from Laboratory Physiological Chemistry, Yale University, vol. III, pp. 19–38.

The Influence of Urethan, Paraldehyde, Antipyrin, and Antifebrin on Proteid Metabolism. By R. H. CHITTENDEN. From experiments made by N. P. Washburn, J. E. Dockendorff, H. F. Adams, and H. C. Taylor. Transactions Connecticut Academy of Arts and Sciences, vol. VIII, pp. 39–59. Studies from Laboratory Physiological Chemistry, Yale University, vol. III, pp. 39–59.

The Influence of several New Therapeutic Agents on Amylolytic and Proteolytic Action. By R. H. CHITTENDEN and C. W. STEWART. Transactions Connecticut Academy of Arts and Sciences, vol. VIII, pp. 60-65. Studies from Laboratory Physiological Chemistry, Yale University, vol. III, pp. 60–65.

Caseoses, Casein-Dyspeptone, and Casein-Peptone. By R. H. CHITTENDEN. From experiments made by C. A. Tuttle and Charles Norris, Jr. Transactions Connecticut Academy of Arts and Sciences, vol. VIII, pp. 66–105. Studies from Laboratory Physiological Chemistry, Yale University, vol. III, pp. 66–105.

Some Experiments on the Influence of Arsenic and Antimony on Glycogenic Function and Fatty Degeneration of the Liver. By R. H. CHITTENDEN and J. A. BLAKE. Transactions Connecticut Academy of Arts and Sciences, vol. VIII, pp. 106–114. Studies from Laboratory Physiological Chemistry, Yale University, vol. III, pp. 106–114.

The Nature and Chemical Composition of the Myosin of Muscle Tissue. By R. H. CHITTENDEN and G. WYCKOFF CUMMINS. Transactions Connecticut Academy of Arts and Sciences, vol. VIII, pp. 115–138. Studies from Laboratory Physiological Chemistry, Yale University, vol. III, pp. 115–138.

Myosinoses. By W. KÜHNE and R. H. CHITTENDEN. Transactions Connecticut Academy of Arts and Sciences, vol. VIII, pp. 139–147. Studies from Laboratory Physiological Chemistry, Yale University, vol. III, pp. 139–147.

Myosin und Myosinosen. By W. Kühne and R. H. Chittenden. Zeitschrift für Biologie, Band XXV, pp. 358–367.

The Relative Absorption of Nickel and Cobalt. By R. H. Chittenden and Charles Norris, Jr. Transactions Connecticut Academy of Arts and Sciences, vol. VIII, pp. 148–157. Studies from Laboratory Physiological Chemistry, Yale University, vol. III, pp. 148–157.

1889 Observations on the Digestive Ferments. By R. H. Chittenden. Medical News, February.

1890 Ueber das Neurokeratin. By W. Kühne and R. H. Chittenden. Zeitschrift für Biologie, Band XXVI, pp. 291–323.

On the Primary Cleavage Products formed in the Digestion of Gluten-Casein of Wheat by Pepsin-Hydrochloric Acid. By R. H. Chittenden and E. E. Smith. The Journal of Physiology, vol. XI, pp. 410–434.

Crystalline Globulin and Globuloses, or Vitelloses. By R. H Chittenden and John A. Hartwell. The Journal of Physiology, vol. XI, pp. 435–447.

1891 The Relative Formation of Proteoses and Peptones in Gastric Digestion. By R. H. Chittenden and J. A. Hartwell. The Journal of Physiology, vol. XII, pp. 12–22.

The Primary Cleavage Products formed in the Digestion of Gelatin. By R. H. Chittenden and Fred. P. Solley. The Journal of Physiology, vol. XII, pp. 23–33.

Myosin-Peptone. By R. H. Chittenden and Ralph S. Goodwin. The Journal of Physiology, vol. XII, pp. 34–41.

The Nutritive Value of Beef Preparations. By R. H. Chittenden. Medical News, vol. LVIII, pp. 716–719.

The Influence of Alcohol on Proteid Metabolism. By R. H. Chittenden. From experiments conducted by Charles Norris, Jr., and E. E. Smith. The Journal of Physiology, vol. XII, pp. 220–232.

A Study of the Proteids of the Corn, or Maize, Kernel. By R. H. Chittenden and Thomas B. Osborne. American Chemical Journal, vol. XIII, pp. 453–468, 529–552; vol. XIV, pp. 20–44.

On the Ferments contained in the Juice of the Pineapple (*Ananassa sativa*), together with some Observations on the Composition and Proteolytic Action of the Juice. By R. H. Chittenden, E. P. Joslin, and F. S. Meara. Transactions Connecticut Academy of Arts and Sciences, vol. VIII, 28 pp.

1892 A Case of Alleged Naphtha Poisoning in a Rubber Factory, with an Inquiry into the Effects of the Inhalation of Naphtha Vapor. By R. H. CHITTENDEN and JOHN W. FARLOW. Boston Medical and Surgical Journal.

Papoid-Digestion. By R. H. CHITTENDEN. Transactions Connecticut Academy of Arts and Sciences, vol. IX, 36 pp.

1893 A Comparison of Artificial and Natural Gastric Digestion, together with a Study of the Diffusibility of Proteoses and Peptone. By R. H. CHITTENDEN and GEORGE L. AMERMAN. The Journal of Physiology, vol. XIV, pp. 483-508.

1894 On the Proteolytic Action of Bromelin, the Ferment of Pineapple Juice. By R. H. CHITTENDEN. From experiments made by Theodore S. Hart, Theodore C. Janeway, W. H. P. Bronson, and Vertner Kenerson. The Journal of Physiology, vol. XV, pp. 249-310.

A Study of the Primary Cleavage Products resulting from the Action of Superheated Water on Coagulated Egg-Albumin. By R. H. CHITTENDEN and FRANK S. MEARA. The Journal of Physiology, vol. XV, pp. 501-534.

On the Proteolysis of Crystallized Globulin. By R. H. CHITTENDEN and LAFAYETTE B. MENDEL. The Journal of Physiology, vol. XVII, pp. 48-80.

1896 A Study of some Infant Foods in comparison with Mother's Milk. By R. H. CHITTENDEN. New York Medical Journal, pp. 71-75.

The Influence of Alcohol and Alcoholic Drinks upon the Chemical Processes of Digestion. By R. H. CHITTENDEN and LAFAYETTE B. MENDEL. American Journal of Medical Science, January-April, 86 pp.

1897 The Mucin of White Fibrous Connective Tissue. By R. H. CHITTENDEN and WILLIAM J. GIES. The Journal of Experimental Medicine, vol. I, pp. 186-201.

The Rotary Properties of some Vegetable Proteids. By ARTHUR C. ALEXANDER. The Journal of Experimental Medicine, vol. I, pp. 304-322.

The Gelatin from White Fibrous Connective Tissue. By WILLARD G. VAN NAME. The Journal of Experimental Medicine, vol. II, pp. 117-129.

1898 The Influence of Borax and Boric Acid upon Nutrition, with Special Reference to Proteid Metabolism. By R. H. CHITTENDEN and WILLIAM J. GIES. American Journal of Physiology, vol. I, pp. 1-39.

A Further Study of the Influence of Alcohol and Alcoholic Drinks upon Digestion, with Special Reference to Secretion. By R. H. CHITTENDEN, LAFAYETTE B. MENDEL, and HOLMES C. JACKSON. American Journal of Physiology, vol. I, pp. 164–209.

The Chemical Composition and Nutritive Value of some Edible American Fungi. By LAFAYETTE B. MENDEL. American Journal of Physiology, vol. I, pp. 225–238.

Papain-Proteolysis, with some Observations on the Physiological Action of the Products formed. By R. H. CHITTENDEN, LAFAYETTE B. MENDEL, and H. E. McDERMOTT. American Journal of Physiology, vol. I, pp. 255–276.

The Influence of Bile and Bile Salts on Pancreatic Proteolysis. By R. H. CHITTENDEN and ALICE H. ALBRO. American Journal of Physiology, vol. I, pp. 307–335.

Notes on Cetraria Islandica (Iceland Moss). By ERNEST W. BROWN. American Journal of Physiology, vol. I, pp. 455–460.

Variations in the Amylolytic Power and Chemical Composition of Human Mixed Saliva. By R. H. CHITTENDEN and A. N. RICHARDS. American Journal of Physiology, vol. I, pp. 461–476.

On the Excretion of Kynurenic Acid. By LAFAYETTE B. MENDEL and HOLMES C. JACKSON. American Journal of Physiology, vol. II, pp. 1–28.

1899 On the Paths of Absorption for Proteids. By LAFAYETTE B. MENDEL. American Journal of Physiology, vol. II, pp. 137–141.

A Chemico-Physiological Study of certain Derivatives of the Proteids. By R. H. CHITTENDEN, LAFAYETTE B. MENDEL, and YANDELL HENDERSON. American Journal of Physiology, vol. II, pp. 142–181.

The Formation of Melanins or Melanin-like Pigments from Proteid Substances. By R. H. CHITTENDEN and ALICE H. ALBRO. American Journal of Physiology, vol. II, pp. 291–305.

A Note on the Cholesterin-esters of Birds' Blood. By ERNEST W. BROWN. American Journal of Physiology, vol. II, pp. 306–309.

Zur Kenntniss des durch Säuren abspaltbaren Stickstoffes der Eiweisskörper. By YANDELL HENDERSON. Zeitschrift für physiologischen Chemie, Band XXIX, pp. 47–58.

On Absorption from the Peritoneal Cavity. By LAFAYETTE B. MENDEL. American Journal of Physiology, vol. II, pp. 342–351.

Metabolism in the Submaxillary Gland during Rest and Activity. By YANDELL HENDERSON. American Journal of Physiology, vol. III, pp. 19–25.

1900   Observations on the Nitrogenous Metabolism of the Cat, especially on the Excretion of Uric Acid and Allantoin. By LAFAYETTE B. MENDEL and ERNEST W. BROWN. American Journal of Physiology, vol. III, pp. 261–270.

On the Occurrence of Iodine in the Thymus and Thyroid Glands. By LAFAYETTE B. MENDEL. American Journal of Physiology, vol. III, pp. 285–290.

On Uric Acid Formation after Splenectomy. By LAFAYETTE B. MENDEL and HOLMES C. JACKSON. American Journal of Physiology, vol. IV, pp. 163–169.

On the Phosphorus Content of the Paranuclein from Casein. By HOLMES C. JACKSON. American Journal of Physiology, vol. IV, pp. 170–177.

Brief Contributions to Physiological Chemistry. Communicated by LAFAYETTE B. MENDEL. I. On the Occurrence of Iodine in Corals, by LAFAYETTE B. MENDEL, p. 243. II. Glycogen Formation after Inulin Feeding, by R. NAKASEKO, p. 246. III. The Influence of Acids on the Amylolytic Action of Saliva, by G. A. HANFORD, p. 250. IV. On the Connective Tissue in Muscle, by J. H. GOODMAN, p. 260. American Journal of Physiology, vol. IV, pp. 243–263.

STUDIES IN
PHYSIOLOGICAL CHEMISTRY

THE INFLUENCE OF BORAX AND BORIC ACID UPON NUTRITION, WITH SPECIAL REFERENCE TO PROTEID METABOLISM.*

By R. H. CHITTENDEN AND WILLIAM J. GIES.

IN view of the widespread use of borax and boric acid as food preservatives it is somewhat singular that our knowledge of the influence of these substances upon the nutritional processes of the body is so slight and uncertain. E. de Cyon,[†] M. Gruber,[‡] and J. Forster [§] have indeed studied the action of these agents upon proteid metabolism, but with results which are utterly lacking in harmony. Thus Cyon's work with borax seemingly indicates that proteid metabolism is diminished under its influence, *i. e.*, that borax tends to protect the consumption of proteid matter in the tissues. Gruber's experiments, on the other hand, indicate with equal positiveness that borax has no proteid sparing power, but really leads to an increase in the rate of proteid metabolism. To add to the uncertainty, the experiments with boric acid carried out under Forster's supervision tend to show that this agent is wholly without influence upon proteid metabolism. Obviously,

---

* Reprinted from the American Journal of Physiology, vol. i.

† Cyon, Sur l'action physiologique du borax. Comptes rendus, 1878, tome 87, p. 845.

‡ Gruber, Ueber den Einfluss des Borax auf die Eiweisszersetzung im Organismus. Zeitschr. f. Biol., 1880, Band 16, p. 198.

§ Forster, Ueber die Verwendbarkeit der Borsäure zur Conservirung von Nahrungsmitteln. Nach Versuchen von Dr. G. H. Schlencker aus Surakarta. Archiv f. Hygiene, 1884, Band 2, p. 75.

conclusions which are so much at variance cannot be accepted without careful consideration.

Cyon's experiments were conducted simultaneously on three full-grown dogs which were fed upon a diet almost exclusively proteid. His observations were practically limited to determining changes in body-weight during short periods, with an estimation of the nitrogen of the urine. He found that during the period when borax was included in the food, the animals gained noticeably in body-weight and that less nitrogen was contained in the excreta than in the ingesta. From these very crude observations the conclusion was drawn that borax, even to the extent of 12 grams per day, may be ingested with the food, especially when the latter is essentially proteid in nature, without provoking the slightest disturbance in general nutrition. Further, Cyon appeared to see in his results evidence that borax, if substituted for common salt in food, will facilitate the assimilation of the latter and bring about a great increase in the weight of the animal. Such deductions, however, were wholly unwarranted from the data at hand, for not only were the periods of observation exceedingly short, but, as pointed out by both Gruber * and C. Voit,† the animals at the beginning were much emaciated and received throughout the experiment such excessive quantities of meat that increase of body-weight would have inevitably followed without the presence of borax. Consequently, all that can be inferred legitimately from Cyon's experiments is that assimilation and general metabolism were not seriously affected by borax in the quantities given.

In Gruber's work more scientific methods were pursued, but it may well be questioned whether the conditions under which the experiments were conducted were adapted for bringing out clearly the full action of borax upon proteid metabolism. The two dogs employed were fed simply upon meat and water, and were presumably in a condition of nitrogenous equilibrium. In the first experiment, where the

* Gruber, loc. cit.
† Voit, Hermann's Handbuch der Physiologie, Band 6, Theil I, p. 165.

animal received daily 1500 grams of meat and 200 c.c. of water, the daily excretion of urea in the urine varied from 75.82 grams to 110.30 grams during the six days prior to the administration of borax. Then 20 grams of borax were introduced with the food, an amount so large that vomiting was at once produced, leading to a loss of about 5 grams of the borax and about 100 grams of the meat, with most of the water. On this day, however, 108.20 grams of urea were excreted in the urine, although the food consumed was 100 grams less than the usual quantity. On the two following days, without borax and with the full complement of food, the excretion of urea amounted to 109.00 and 107.60 grams respectively. From these results Gruber concludes that the borax increased the excretion of urea 4–6 per cent. In the second experiment, with a dog of 34 kilos body-weight, fed on a daily ration of 1100 grams of meat and 200 c.c. of water, the daily excretion of urea varied from 70.86 grams to 80.60 grams for the four days of the normal period, while the administration of 10 grams of borax was accompanied by an excretion of 82.14 grams of urea, and, on the second day following, the introduction of 20 grams of borax was accompanied by an excretion of 85.25 grams of urea. Further, on this latter day the volume of urine rose to 1310 c.c., while the largest daily excretion prior to this day was 1040 c.c. Gruber, therefore, concludes that borax does not spare proteid as Cyon asserts, but, just as in the case of common salt, sodium sulphate, and other neutral salts, it causes an increase in the elimination of water from the body and induces therewith an increased proteid catabolism. It is not to be inferred from this statement that there is simply an increased washing out of urea from the tissues, for, as Voit * has pointed out, the amounts of urea excreted on the days following the ingestion of borax simply fall back to the neighborhood of the average for the normal period, and do not drop below that average. Gruber also concludes that borax has no unfavorable influence upon the assimilation of food, since the quantity of fæces,

* Voit, loc. cit., p. 165.

their content of solid matter and of nitrogen are within the
limits of the normal elimination during periods when meat
alone is fed. Further, no harmful influence, even after the
ingestion of the largest dose — 20 grams — was to be observed,
and the appetite of the animal was found to be undiminished
on the days following that upon which borax was given.
The objection we would make to accepting Gruber's conclu-
sions in their entirety is that they are based solely upon the
results following the administration of two large doses of
borax, 10 and 20 grams, whereas, to our mind, longer periods
with a dosage of borax continued for several days in succession
would seemingly render the conditions much more favorable
for an accurate judgment as to the character of the influence
exerted by the substance on tissue changes. Further, since
urea alone was determined in the urine, possible minor changes
connected with the presence of the salt would naturally be
overlooked. Lastly, we are inclined to the view that it is
extremely hazardous to draw such sweeping conclusions from
one or two single experiments of this nature, especially where,
as in the animal body, individual characteristics not infrequently
give rise to exceptional results quite foreign to those ordinarily
obtainable.

In Forster's work with boric acid, Dr. Schlencker experi-
mented on himself, using a mixed diet and taking boric acid
in daily doses of 1–3 grams. Each experiment consisted of
three periods, of three days each, the boric acid being taken
in the middle period. The conclusions arrived at were that
proteid metabolism is not influenced, the excretion of urea in
the boric-acid period being midway between that of the fore
and after periods. It was noticed, however, that the quantity
of ethereal sulphuric acid in the urine was considerably
lessened in the boric-acid period and in the period following,
thus implying an inhibitory influence upon the putrefactive
processes of the intestine. Further, it was observed that the
volume of the fæces, together with the contained nitrogen,
was greatly increased under the influence of boric acid, from
which it was inferred that this agent interferes with the

assimilation of the food and perhaps, at the same time, gives rise to an increased secretion of mucus with a possible increase in the discharge of epithelial cells from the intestinal mucosa. This latter, however, is purely conjectural. Increased secretion of bile is also said to result from the action of boric acid. On the pulse and temperature no action was observed.

It is thus quite evident that the influence of borax and boric acid on nutrition, and especially their influence on proteid metabolism, is by no means wholly settled. The preceding statements clearly emphasize the uncertainty of our present information on the more essential features of the question before us, and we have therefore deemed it desirable to carry out, as thoroughly as possible, a series of experiments upon the action of both borax and boric acid on proteid metabolism and related phases of nutrition.

Conduct of the Experiments. — The experiments were conducted wholly upon full-grown dogs ranging in weight from 8 to 12 kilos. The animals were confined in suitable cages partially lined with galvanized iron and with the floor so arranged that both fluid and solid excreta could be collected in their entirety, while the upper portions of the cage were so constructed as to permit unrestricted circulation of air. In view of the length of the experiments — ranging from twenty-seven to fifty-six days each, with periods of eight to ten days' duration — it seemed inadvisable as well as unnecessary to empty the bladder each day with a catheter. Such diurnal variations as might possibly occur from incomplete emptying of the bladder at the end of the twenty-four hours would obviously be neutralized in periods of the above length, and consequently the urine was collected as naturally excreted, thus avoiding any possible disturbance of the normal condition of the bladder, etc. At the end of each twenty-four hours, the urine collected was combined, and its volume, specific gravity, etc., determined, after which the bottom of the cage was rinsed with a little distilled water and these washings added to the main fluid. The latter was then made up to some convenient volume in preparation for the daily analysis.

The fæces whenever passed were collected in a weighed dish, the mass thoroughly desiccated over a water-bath, and the dry weight ascertained. The dried material was then pulverized and the nitrogen-content as well as the ether-soluble matter determined in sample portions. The nitrogen determinations were always made in duplicate by the Kjeldahl method and rarely varied more than 0.05 per cent. Whenever, as sometimes occurred, hair accumulated in the cage it was likewise collected and the nitrogen determined. The ether-soluble matter was determined by extraction of the dried fæces in a Soxhlet-apparatus.

The animals were fed during the experiments on a mixed diet composed of fresh lean beef, cracker dust, lard, and water. The meat was prepared as follows: fresh lean beef, freed as far as possible from all adherent fat and connective tissue, was run through a hashing machine, after which it was enclosed in a bag of thin cloth, placed under a heavy press, and kept there under increasing pressure for several hours, the bloody fluid which drained off being thrown away. By this method there results a mass of tissue free from surplus moisture, and which, when enclosed in a bottle, will keep perfectly fresh on ice for seven to ten days without separation of fluid. Several advantages accrue from this method. Thus, we have a perfectly homogeneous mixture which can be drawn from for at least a week with surety that its nitrogen-content is constant. There is therefore no necessity for a daily determination of nitrogen in this portion of the diet, for each sample can be analyzed when prepared and the data accepted as long as the meat keeps fresh. Further, meat prepared in this manner at different times, if subjected to essentially the same pressure, varies only slightly in its content of nitrogen. We have invariably analyzed each lot when prepared to avoid any possibility of error, but, as the following results show, the differences in composition are very slight and necessitate very little alteration in the proportion of meat when changing from one lot to another. The following results are a few of the many obtained:

	Weight of Meat.	Absolute Content of Nitrogen.	Percentage of Nitrogen.
	grm.	grm.	
1	0.8703	0.03041	3.49
	0.7710	0.02682	3.48
	0.7631	0.02628	3.44
2	0.7673	0.02716	3.54
	0.9228	0.03238	3.51
	1.0591	0.03723	3.52
3	0.8478	0.03015	3.56
	1.0014	0.03591	3.59
	0.8876	0.03152	3.55
4	1.0082	0.03642	3.61
	1.0445	0.03783	3.62
	1.0803	0.03961	3.67
5	1.1977	0.04265	3.56
	0.8142	0.02902	3.56
	0.9793	0.03463	3.54

The carbohydrate element in the diet, as already stated, was supplied by commercial cracker dust. This was purchased in large quantity and preserved in well stoppered bottles. It contained on an average 1.46 per cent of nitrogen. The lard employed was entirely free from any recognizable amount of nitrogen.

The daily diet was divided into two equal portions, one-half being fed at 8 A.M. and the other half at 6 P.M. When borax or boric acid was given, the daily dose was likewise divided and given either with the food or directly after. The body-weight of the animal was taken each morning just before feeding. Each day's urine included the fluid passed from 8 A.M. to 8 A.M. of the next day.

Methods of Analysis. — Nitrogen was determined wholly by the Kjeldahl method, viz., in the daily analyses of the urine, fæces, and food material. All analyses were made in duplicate, and the figures given are based upon the average of closely agreeing results. In analysis of the urine 5 c.c. were used for each determination, oxidation being carried out in a long-necked Kjeldahl flask with 10 c.c. of sulphuric acid and a crystal of cupric sulphate, thus doing away with the necessity of adding sodium sulphide in the distillation. The ammonia

formed was distilled into quarter-normal hydrochloric acid, the latter being titrated with quarter-normal ammonia, using congo red as an indicator.

Sulphur and phosphorus were determined in the customary manner by evaporating a given volume of the urine — 25 c.c. for each determination — in a roomy silver crucible with 10 grams of pure sodium hydroxide (made from the metal) and 2 grams of potassium nitrate, igniting the residue until oxidation was complete and treating the fused mass with water. For sulphur, the mixture was acidified with hydrochloric acid, evaporated to dryness, the residue moistened with a few drops of hydrochloric acid and dissolved in hot water. The filtered solution was then precipitated in the usual manner with barium chloride, the resultant barium sulphate filtered, ignited, and weighed, thus giving data for calculation of the total sulphur. For phosphorus, the aqueous extract of the oxidized urine was acidified with nitric acid, evaporated to dryness, the residue moistened with nitric acid and dissolved in warm water. From this solution the phosphoric acid was precipitated in the usual manner with molybdic solution and eventually transformed into ammonio-magnesium phosphate. From the weight of magnesium pyrophosphate obtained, the total phosphorus of the urine was calculated.

Uric acid was determined by the well-known Salkowski-Ludwig silver method, using 100–200 c.c. of urine.

Phosphoric acid was determined by Mercier's * modification of Neubauer's method, *i. e.*, by titration of 50 c.c. of urine with a standard solution of uranium nitrate and tincture of cochineal as an indicator.

Total sulphuric acid was estimated by diluting 25 c.c. of urine with 3–4 volumes of water, adding 5 c.c. of dilute hydrochloric acid, heating to boiling, and precipitating hot with barium chloride. The barium sulphate so obtained, after standing twenty-four hours in a warm place, was washed with hot water until free from chlorides and lastly with hot alcohol, ignited, and weighed.

---

* See Neubauer und Vogel's Analyse des Harns, neunte Auflage, p. 450.

Combined sulphuric acid was determined by Baumann's method, using 100 c.c. of urine.*

Chlorine was determined in 10 c.c. of urine by Neubauer and Salkowski's modification of Mohr's method.† Other methods occasionally made use of are referred to in their appropriate place.

First Experiment. With Borax.—The animal made use of in this experiment was a short-haired mongrel bitch weighing about 12 kilos. She was brought into a condition approximating to nitrogenous equilibrium only after a preliminary period of nearly three weeks, during which time superfluous fat was lost and she became wholly accustomed to her surroundings. The daily food at the time the experiment actually commenced, consisted of 250 grams of the prepared meat, 70 grams of cracker dust, 40 grams of lard, and 500 c.c. of water. It contained 9.814 grams of nitrogen. This diet, with the above content of nitrogen, was adhered to throughout the entire experiment of twenty-seven days, the only variation being the slight changes in the amount of nitrogen, to be seen in the tables, incidental to the use of different lots of meat and in the employment of gelatin capsules during the borax period. These gelatin capsules, in which the borax was administered, contained 14.95 per cent of nitrogen, the four capsules used each day during the borax period containing 0.12 gram of nitrogen. This amount was naturally included in the nitrogen of the food.

The experiment extended through twenty-seven days and was divided into three periods of nine days each : a fore or normal period during which no borax was given, a borax period during which 45 grams of borax (5 grams a day) were administered, and an after period when normal conditions again prevailed. During the borax period of nine days the quantity of borax given per day amounted to nearly 0.6 per cent of the total food and drink ingested, while of the solid food it formed 1.3 per cent. This dosage of borax, considering the size of the animal, was fairly large, and with this

* Ibid., p. 447.   † Ibid. p. 437.

particular dog considerable difficulty was experienced in inducing the animal to take it. At first the borax was simply mixed with the food, but its presence was quickly detected and the food refused, although it was eventually coaxed down, but with some difficulty. After this first day the borax was given in capsules, as already stated, and no further difficulty of this sort was experienced. Three times during the borax period, however, the animal was nauseated and vomited a portion of the food, thus showing that this quantity of borax was sufficient to disturb the physiological equilibrium of the animal. The vomited matter was eventually eaten, however, later in the day, so that this occurrence did not disturb the validity of the experiment. It will be remembered that in Gruber's experiment with a much larger dog (39 kilos) 20 grams of borax likewise caused vomiting. In his experiment, however, the entire dose of borax was taken at one time, while in our case, 2.5 grams were given in the morning and a like quantity at night. Hence, taking into account the weight of the dog, it might perhaps be argued that 0.25 gram of borax to 1 kilo of body-weight will produce vomiting. This, however, is very questionable, for in the above experiment the dog did not vomit until the afternoon of December 5, when she had already taken 12.5 grams of borax. In other words, the animal was without doubt suffering in part from the cumulative action of the salt. Thus, there was a slight attack of vomiting again on the fifth day (December 7) and a final attack on the eighth day (December 10). During the after period of nine days the animal was perfectly normal, and at the close of the period, to again test the action of the borax, 5 grams were given at one time shortly after the morning meal. Forty-five minutes afterwards the animal vomited, and this occurred three times during the forenoon. We are inclined to lay particular emphasis upon this action of the borax because it tends to show that in this first experiment the dosage of borax through the nine days' period was as large as it well could be for this particular animal without vitiating the experiment, and that

the conditions were therefore well adapted for bringing out distinctly any possible influence the borax might have upon the metabolic phenomena of the body. Further, we would call attention to the obvious advantage — in spite of the greater labor involved — of continuing experiments of this character over comparatively long periods of time. To be sure, in some cases where the substance being tested has a marked physiological action, a single dose may show at once the character of the influence exerted, but too often erroneous conclusions are arrived at through negligence of this precaution. Where, however, the substance under examination is given for five to ten days consecutively, with careful examination of the excreta, the chances of detecting minor influences are greatly increased, and at the same time the danger of being led astray by a single exceptional result — or by other possible errors — is greatly diminished.

The tables on the three following pages contain the analytical results obtained throughout the experiment.

On referring to the tables containing the results of the first experiment, it is to be noted that in the fore period of nine days the total nitrogen ingested amounted to 88.326 grams, while in the urine excreted during this period there were contained 87.185 grams of nitrogen, and in the fæces 2.122 grams, making a total of 89.307 grams of nitrogen; hence the nitrogen balance for the period of nine days is −0.981 gram. The body-weight remained practically constant. The slight excess of nitrogen excreted over the amount ingested in this period is due possibly to lack of complete involution of the mammary glands; [*] the deficiency, however, is too slight, considering the length of the period, to need much consideration. For comparison, the results of the three periods showing the relative excretion of nitrogen, may be arranged in tabular form .

[*] MARCUSE: Ueber den Nährwerth des Cäseïns, Pflüger's Archiv f. d. ges. Physiol., 1896, Band 64, p. 223.

FIRST EXPERIMENT.—FORE PERIOD.

Date. 1896.	Body. Weight.	Food. Nitrogen.	Urine Vol.	Sp. gr.	Reaction (litmus.)	Nitrogen.	Uric Acid.	Phos-phorus.	Sulphur.	Total SO₃.	Combined SO₃.	Faeces. Dry Weight.	Faeces. Nitrogen.
	kilos.	grm. 9.814	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
Nov. 24	10.9	9.814	505	1018	Acid.	7.945	0.038	0.468	0.491	0.962	0.068	:	:
25	10.9	9.814	716	1018	Acid.	11.861	0.049	0.646	0.720	1.388	0.075	:	:
26	10.9	9.814	773	1017	Acid.	11.367	0.061	0.688	0.671	1.843	0.077	:	:
27	11.0	9.814	786	1016	Acid.	12.476	0.049	0.768	0.737	1.621	0.084	:	:
28	11.0	9.814	650	1017	Acid.	10.069	0.047	0.585	0.586	1.214	0.064	:	:
29	11.0	9.814	415	1017	Acid.	6.102	0.040	0.325	0.381	0.765	0.032	:	:
30	10.8	9.814	770	1019	Acid.	12.302	0.066	0.760	0.758	1.554	0.084	:	:
Dec. 1	10.9	9.814	575	1017	Acid.	8.995	0.040	0.505	0.570	1.148	0.062	:	:
2	11.0	9.814	489	1018	Acid.	6.568	0.038	0.410	0.405	0.804	0.055	88.15	2.122
Totals		88.326	5629		Nitrogen of Urine = 87.185 Nitrogen of Faeces = 2.122	89.307	0.428	5.150	5.319	10.699	0.591	88.15	2.122
Daily Averages		9.814	625			9.923	0.048	0.572	0.591	1.189	0.066		0.236

FIRST EXPERIMENT.—BORAX PERIOD.

Date. 1896.	Body. Weight.	Food. Nitrogen.	Borax.	Urine. Vol.	Sp. gr.	Reaction (Litmus).	Nitrogen.	Uric Acid.	Phosphorus.	Sulphur.	Total SO₃.	Combined SO₃.	Faeces. Dry Weight.	Nitrogen.
	kilos.	grm.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
Dec. 3	10.9	9.903	5	796	1021	Acid.	13.344	0.054	0.821	0.789	1.631	0.097	. . .	. . .
4	11.1	9.933	5	368	1022	Alkaline.	5.909	0.032	0.321	0.371	0.705	0.039	. . .	. . .
5	11.2	9.983	5	485	1025	Alkaline.	9.188	0.039	0.535	0.527	1.103	0.057	. . .	. . .
6	11.1	9.988	5	520	1027	Alkaline.	10.043	0.042	0.568	0.592	1.197	0.060	. . .	. . .
7	11.1	10.016	5	686	1024	Alkaline.	12.823	0.050	0.818	0.754	1.526	0.068	85.91	2.292
8	11.2	10.100	5	422	1024	Alkaline.	7.412	0.051	0.444	0.426	0.825	0.042	. . .	. . .
9	11.2	10.100	5	604	1023	Alkaline.	10.742	0.049	0.615	0.596	1.228	0.069	. . .	. . .
10	11.3	10.100	5	498	1026	Alkaline.	9.846	0.081	0.621	0.600	1.174	0.060	. . .	. . .
11	11.3	10.100	5	602	1020	Alkaline.	8.825	0.063	0.456	0.554	1.040	0.063	24.68	1.627
						Nitrogen of Urine = 88.127 Nitrogen of Faeces = 3.919								
Totals	. . .	90.118	45	4981			92.046	0.411	5.099	5.209	10.429	0.555	60.59	3.919
Daily Averages	. . .	10.018	5	553			10.227	0.046	0.567	0.579	1.159	0.062		0.435

FIRST EXPERIMENT. — AFTER PERIOD.

Date. 1896.	Body. Weight.	Food. Nitrogen.	Urine. Vol.	Sp. gr.	Reaction. (litmus.)	Nitrogen.	Uric Acid.	Phos-phorus.	Sulphur.	Total SO₃.	Combined SO₃.	Faeces. Dry Weight.	Nitrogen.
	kilos.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
Dec. 12	11.5	9.981	488	1019	Acid.	8.727	0.042	0.441	0.596	1.024	0.055	...	...
13	11.3	9.981	670	1018	Acid.	10.632	0.053	0.589	0.716	1.247	0.073	...	...
14	11.4	9.981	691	1017	Acid.	10.047	0.039	0.621	0.742	1.265	0.083	...	...
15	11.5	9.981	551	1016	Acid.	7.804	0.032	0.482	0.601	0.978	0.049	...	...
16	11.4	9.981	681	1018	Acid.	10.549	0.061	0.694	0.736	1.345	0.073	33.25	1.995
17	11.5	9.981	595	1019	Acid.	10.121	0.036	0.602	0.662	1.213	0.062	...	...
18	11.5	9.981	572	1018	Acid.	9.232	0.036	0.587	0.613	1.119	0.069	...	...
19	11.5	9.981	630	1017	Acid.	9.587	0.056	0.574	0.610	1.083	0.068	...	...
20	11.5	10.086	549	1019	Acid.	9.678	0.044	0.574	0.616	1.180	0.069	25.45	1.629
					Nitrogen of Urine = 86.377								
					Nitrogen of Faeces = 3.624								
						90.001							
						10.000							
Totals		89.884	5427				0.389	5.224	5.892	10.454	0.601	58.70	3.624
Daily Averages		9.987	603				0.043	0.580	0.655	1.162	0.067		0.408

FIRST EXPERIMENT. — GENERAL SUMMARY.

Periods.	Total Nitrogen. Ingested.	Excreted.	Balance.	Urine. Vol.	Nitrogen.	Uric Acid.	Phos-phorus.	Sulphur.	Total SO₃.	Combined SO₃.	Faeces. Dry Weight.	Nitrogen.
	grm.	grm.	grm.	c.c.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
Fore . . .	88.326	89.307	−0.981	5629	87.185	0.428	5.150	5.319	10.699	0.591	38.15	2.122
Borax . .	90.118	92.046	−1.928	4981	88.127	0.411	5.099	5.209	10.429	0.555	60.59	3.919
After . . .	89.884	90.001	−0.117	5427	86.377	0.389	5.224	5.892	10.454	0.601	58.70	3.624

	Fore Period.	Borax Period.	After Period.
Nitrogen of Food . . .	88.326	90.118	89.884
Nitrogen of Urine . . .	87.185 ⎫ 89.307	88.127 ⎫ 92.046	86.377 ⎫ 90.001
Nitrogen of Fæces . . .	2.122 ⎭	3.91 ⎭	3.624 ⎭
Nitrogen Balance . .	−0.981	−1.928	−0.117
Ratio of Urine Nitrogen to Food Nitrogen . .	98.6 per cent.	97.7 per cent.	96.0 per cent.

It is thus evident that in this experiment, in spite of the large doses of borax and the length of the period, proteid metabolism is not modified in any noticeable degree. The amount of nitrogen eliminated through the urine in proportion to the nitrogen of the food, during the borax period, differs from that of the fore period only to a slight extent, and this difference is due apparently to a diminished assimilation of the proteid food. The change in the nitrogen balance of the borax period is plainly caused by a slight increase in the amount of fæcal nitrogen, and not to increased metabolism, thus indicating that the borax has a tendency to diminish somewhat the absorption of proteid food, or possibly leads to an increased secretion of mucus. When, however, the nitrogen of the fæces of the borax period is compared with both that of the fore and after periods the increase is seen to be so slight that it is perhaps unwise to attach much importance to it. Certainly the borax, though given in doses sufficiently large to keep the animal on the verge of nausea, did not in this experiment interfere greatly with the digestion of any of the food-stuffs, since the fæces of the borax period are not much greater in amount than those of the after period, though somewhat larger than those of the fore period.

The weight of the animal during the twenty-seven days' period showed a tendency to rise somewhat, *i. e.*, from 10.9 kilos to 11.5 kilos. This, however, is not to be attributed to a laying-on of fat nor to a retention of nitrogenous matter by the body, but is the result simply of a diminished excretion of water due to the presence of the borax. The results in this connection are in direct opposition to those obtained by

Gruber with single doses of borax. There is here no suggestion whatever of an increased excretion of water, but, on the contrary, a very marked decrease. Thus, by reference to the accompanying tables, it will be observed that during the fore period the total volume of urine excreted amounted to 5629 c.c. and the body-weight remained practically constant, *i. e.*, 10.9-11.0 kilos. During the borax period, however, the volume of urine excreted fell to 4981 c.c. and the body-weight gradually rose to 11.3 kilos, while in the after period the volume of urine rose to 5427 c.c. with a constant body-weight of 11.5 kilos. It is thus quite clear that borax may decidedly check the output of water through the kidneys, and lead, as in this case, to its retention within the body.

Very noticeable also, in this experiment, is the sudden change in the specific gravity of the urine, as also in the reaction of the fluid, when borax is given. Thus, in the fore period the specific gravity of the urine stood at 1017-1018, but at the opening of the borax period it rose at once to 1022-1027, dropping back, however, as the borax was discontinued. Similarly, the reaction of the normal urine was acid to litmus, but on exhibition of borax, the reaction quickly changed to alkaline. The marked rise in the specific gravity of the urine during the borax period is not due solely to diminished elimination of water nor to increase in the proportion of metabolic products, but mainly to the borax itself, which is rapidly eliminated through the urine. We have not made any special trial to ascertain how soon the borax appears in the urine after its administration, but we have observed that the urine collected on the first day of the borax period gives, after acidulation with hydrochloric acid, a strong reaction with turmeric paper for boric acid. Further, that the elimination of borax through the urine is very rapid is manifest from the fact that, at the end of the borax period, the animal having received 45 grams of the salt, no trace of a reaction could be obtained with turmeric paper on the *second* day of the after period. In other words, elimination of the borax was practically complete twenty-four to thirty-six hours

after the last dose had been taken. These observations accord with Johnson's statements * that borax and boric acid begin to be eliminated through the urine a short time after their administration.

While it is clear from a study of the nitrogen excretion that proteid metabolism, under the conditions of this experiment, is not materially affected by borax, the other analytical results must not be overlooked. Thus, in the borax period the excretion of phosphorus, sulphur, total sulphuric acid, and combined sulphuric acid is slightly below that of the fore and after periods. The differences, however, are so small that it is perhaps unwise to draw any positive conclusions from them, other than to admit their negative character. It can certainly be asserted with perfect safety that the borax has failed to exert any marked influence upon the excretion of either sulphur or phosphorus. In this connection it will be remembered that Forster † found, on feeding boric acid to man, a marked increase in the output of phosphoric acid. Borax, however, certainly fails to produce any such result, its presence in the body (of the dog) tending on the other hand to reduce the output of phosphorus. Further, it is evident that the slight diminution in the excretion of combined sulphuric acid is not sufficient to indicate any inhibitory influence upon intestinal putrefaction. Lastly, the figures obtained in connection with uric acid are such as to indicate a purely negative action.

Second Experiment. With Boric Acid. — The animal experimented on was a short-haired mongrel bitch weighing 8 kilos. Nitrogenous equilibrium was quickly established on a daily diet composed of 160 grams of the prepared meat, 40 grams of cracker dust, 30 grams of lard, and 400 c.c. of water. This diet contained 6.144 grams of nitrogen and was practically adhered to throughout the experiment. The latter was of thirty days' duration, *i. e.*, three periods of 10 days each.

---

* Johnson, Ueber die Ausscheidung von Borsäure und Borax aus dem menschlichen Organismus. Jahresbericht f. Thierchemie, 1885, p. 235. See also, Vigier, Note préliminaire sur l'action physiologique du borate de soude. Comptes rendus Soc. de Biol. Paris, 1883, p. 44.

† Forster, Archiv f. Hygiene, 1884, Band 2, p. 75.

During the middle, or boric acid period, 1–2 grams of boric acid were given daily mixed with the food, the animal taking it without the slightest reluctance and without any apparent effect upon the appetite. No sign of nausea or vomiting was seen. With 2 grams of boric acid per day the mixture of food and drink contained 0.31 per cent, while the dry food contained 0.86 per cent of boric acid. The total amount of boric acid given during the ten days was 14.5 grams.

During the fore period of ten days the animal received a total of 61.440 grams of nitrogen. The nitrogen excreted through the urine for this period amounted to 58.119 grams, while the fæces contained 3.203 grams, thus making a total of 61.322 grams of nitrogen excreted, with a nitrogen balance of +0.118 gram. Plainly, the animal was in a condition of nitrogenous equilibrium.

The relative excretion of nitrogen for the three periods may be seen in the following table:

	Fore Period.	Boric Acid Period.	After Period.
Nitrogen of Food . . .	61.440	62.032	61.943
Nitrogen of Urine . . .	58.119 ⎱ 61.322	59.600 ⎱ 63.538	58.979 ⎱ 62.923
Nitrogen of Fæces . . .	3.203 ⎰	3.938 ⎰	3.944 ⎰
Nitrogen Balance . .	+0.118	1.506	−0.980
Ratio of Urine Nitrogen to Food Nitrogen . .	94.5 per cent.	96.7 per cent.	95.2 per cent.

From these figures it would appear that there is a slight tendency toward stimulation of proteid metabolism. When it is remembered, however, that the nitrogen balance for the boric acid period, −1.506, is the result of ten days' consecutive feeding with boric acid, it is manifest that the stimulating action is very slight, and our results may perhaps be considered as practically in accord with those reported by Forster, who found that in man on a mixed diet, boric acid in moderate doses (1–3 grams) was without influence on proteid decomposition as measured by the excretion of urea. Upon the assimilation of the proteid food there is no evidence of any action, *i. e.*, the nitrogen content of the fæces during the boric acid

period is essentially the same as that of the fore and after periods. Further, the total weight of fæces for each of the three periods is so nearly the same, it is quite evident that assimilation has not been materially interfered with. In this respect our results fail to agree with those reported by Forster, who found that small doses of boric acid (1 gram in two days) given to a man on a mixed diet, and on a milk and egg diet, increased the excretion of fæces; this increase being due, according to Forster, not to any decrease in the assimilation of fat nor to increase in the volume of the secretions, but to a decreased assimilation of the proteid food under the influence of the boric acid. This difference in our results may of course depend upon the difference in the character of the animal species. In our experiment, the weight of the animal remained perfectly constant throughout the entire period of thirty days.

The accompanying tables contain the various data obtained.

Unlike borax, boric acid fails to produce any change in the volume of the urine. Thus, in the fore period of ten days the total volume excreted amounted to 4647 c.c., while in the boric acid period of the same length the total volume was 4665 c.c., and in the after period 4644 c.c. Further, there is no marked difference, to be measured by litmus paper, in the reaction of the fluid, although, as the tables show, alkaline reaction is more common in the normal periods than in the boric acid period. In the latter period, however, the specific gravity of the urine, as might be expected, shows a higher average than in the two normal periods. This is due, as in the case of borax, to the rapid elimination of the boric acid through the urine. The latter shows the presence of the acid by the turmeric test on the first day of the boric acid period, while on the second day of the after period all trace of a reaction disappears, thus showing that the acid is rapidly eliminated from the body and is practically completely removed twenty-four to thirty-six hours after the last dose.

Upon the elimination of uric acid, boric acid appears to have a slight inhibitory effect, at least under the conditions of this

SECOND EXPERIMENT. — FORE PERIOD.

Date.	Body.	Food.	Urine.									Faeces.	
1897.	Weight.	Nitrogen.	Vol.	Sp. gr.	Reaction (litmus).	Nitrogen.	Uric Acid.	Total SO₃.	Combined SO₃.	Chlorine.	Total P₂O₅.	Dry Weight.	Nitrogen.
	kilos.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
Feb. 24	7.9	6.144	500	1015	Acid.	6.642	0.037	0.682	0.023	0.354	0.960	⋯	⋯
25	7.9	6.144	486	1012	Acid.	5.051	0.055	0.538	0.021	0.404	0.726	⋯	⋯
26	7.9	6.144	460	1014	Acid.	5.741	0.048	0.688	0.029	0.340	0.789	6.96	0.450
27	8.0	6.144	410	1015	Acid.	4.960	0.049	0.560	0.024	0.379	0.665	⋯	⋯
28	7.9	6.144	581	1014	Acid.	7.605	0.096	0.830	0.036	0.573	1.053	⋯	⋯
Mar. 1	8.0	6.144	325	1014	Acid.	4.067	0.033	0.477	0.020	0.210	0.506	⋯	⋯
2	7.9	6.144	525	1016	Alkaline.	7.613	0.052	0.807	0.040	0.404	1.008	11.90	0.780
3	7.9	6.144	440	1014	Alkaline.	5.425	0.057	0.581	0.022	0.407	0.722	10.50	0.657
4	8.0	6.144	370	1014	Alkaline.	4.119	0.026	0.464	0.016	0.291	0.540	⋯	⋯
5	7.9	6.144	550	1015	Acid.	6.900	0.051	0.749	0.026	0.462	1.004	17.30	1.316
					Nitrogen of Urine = 58.119								
					Nitrogen of Faeces = 3.203								
Totals	⋯⋯	61.440	4647			61.322	0.504	6.321	0.257	3.824	7.963	46.66	3.203
Daily Averages		6.144	465			6.182	0.050	0.632	0.026	0.382	0.796		0.820

SECOND EXPERIMENT. — BORIC ACID PERIOD.

Date.	Body.	Food.	Boric Acid.				Urine.						Faeces.	
1897.	Weight.	Nitrogen.		Vol.	Sp. gr.	Reaction (litmus).	Nitrogen.	Uric Acid.	Total SO₃.	Combined SO₃.	Chlorine.	Total P₂O₅.	Dry Weight.	Nitrogen.
	kilos.	grm.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
Mar. 6	7.9	6.144	1	470	1016	Acid.	5.915	0.040	0.678	0.025	0.347	0.911	...	...
7	7.9	6.144	1	505	1016	Acid.	6.390	0.031	0.712	0.032	0.451	0.946	...	...
8	8.0	6.188	1	380	1014	Acid.	4.479	0.028	0.511	0.020	0.259	0.550	10.20	0.710
9	7.9	6.223	1	525	1017	Acid.	7.280	0.041	0.767	0.034	0.856	1.073	...	...
10	8.0	6.223	1.5	400	1016	Alkaline.	4.166	0.026	0.481	0.017	0.388	0.586	9.75	0.660
11	7.9	6.223	1.5	580	1017	Acid.	7.460	0.032	0.808	0.034	0.555	1.017	...	...
12	7.9	6.223	1.5	460	1017	Acid.	6.000	0.040	0.664	0.031	0.538	0.849	16.30	1.317
13	7.9	6.223	2	470	1017	Acid.	6.035	0.041	0.682	0.031	0.565	0.841	...	...
14	7.9	6.223	2	480	1017	Acid.	6.032	0.035	0.648	0.027	0.488	0.828	11.60	0.882
15	7.9	6.223	2	445	1017	Acid.	5.843	0.042	0.683	0.027	0.425	0.899	5.45	0.369
						Nitrogen of Urine = 59.600 Nitrogen of Faeces = 3.938								
Totals	. . .	62.032	14.5	4665			63.538	0.386	6.629	0.278	4.317	8.500	53.30	3.938
Daily Averages		6.203	1.45	467			6.354	0.039	0.663	0.028	0.432	0.850		0.394

SECOND EXPERIMENT.—AFTER PERIOD.

Date. 1897.	Body. Weight.	Food. Nitrogen.	Urine. Vol.	Sp. gr.	Reaction (litmus.)	Nitrogen.	Uric Acid.	Total SO_3.	Combined SO_3.	Chlorine.	Total P_2O_5.	Faeces. Dry Weight.	Nitrogen.
	kilos.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
Mar. 16	7.9	6.223	482	1016	Acid.	6.100	0.057	0.670	0.026	0.346	0.813	5.45	0.369
17	8.0	6.223	360	1014	Alkaline.	4.318	0.028	0.526	0.020	0.226	0.526	7.71	0.476
18	7.9	6.223	560	1016	Alkaline.	7.630	0.096	0.874	0.048	0.604	1.106	...	...
19	7.9	6.223	485	1015	Acid.	6.284	0.052	0.717	0.033	0.448	0.890	8.82	0.655
20	8.0	6.223	425	1018	Alkaline.	4.412	0.040	0.514	0.023	0.366	0.674	...	...
21	7.9	6.223	560	1017	Acid.	7.947	0.069	0.937	0.058	0.742	1.205	12.25	0.814
22	7.9	6.041	490	1015	Alkaline.	5.922	0.044	0.678	0.033	0.528	0.831	...	...
23	7.9	6.188	450	1015	Alkaline.	4.940	0.036	0.575	0.022	0.416	0.739	10.47	0.798
24	7.9	6.188	480	1016	Alkaline.	6.827	0.037	0.749	0.038	0.296	0.979	...	...
25	7.9	6.188	402	1015	Alkaline.	4.599	0.049	0.571	0.020	0.260	0.686	10.90	0.882
					Nitrogen of Urine = 58.979								
					Nitrogen of Faeces = 8.944								
Totals		61.943	4644			62.928	0.508	6.811	0.316	4.282	8.449	55.60	8.944
Daily Averages		6.194	464			6.292	0.061	0.681	0.082	0.428	0.845		0.894

SECOND EXPERIMENT.—GENERAL SUMMARY.

Periods.	Total Nitrogen. Ingested.	Excreted.	Balance.	Urine. Vol.	Nitrogen.	Uric Acid.	Total SO_3.	Combined SO_3.	Chlorine.	Total P_2O_5.	Faeces. Dry Weight.	Nitrogen.
	grm.	grm.	grm.	c.c.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
Fore	61.440	61.322	+0.118	4647	58.119	0.504	6.821	0.267	3.824	7.963	46.66	8.203
Boric Acid .	62.032	63.538	−1.506	4665	59.600	0.386	6.629	0.278	4.317	8.500	53.30	8.938
After	61.943	62.923	−0.980	4644	58.979	0.508	6.811	0.316	4.282	8.449	55.60	8.944

experiment, but upon the excretion of total and combined sulphuric acid, chlorine, and phosphoric acid, no tangible effect is produced. Certainly, the results in connection with combined sulphuric acid do not indicate any retarding effect upon the putrefactive processes of the intestine. In this connection it will be remembered that in Forster's experiments on man doses of boric acid, corresponding to those used by us, apparently gave rise to a marked retardation in the amount of ethereal sulphate excreted. As a result, Forster arrived at the conclusion that boric acid materially reduces intestinal putrefaction. Our results, however, show no action of this kind in the dog, and we are inclined to the view that both borax and boric acid are too rapidly eliminated from the system to be very effective in the intestine. As already stated, the elimination of borax and boric acid through the urine commences almost immediately after their ingestion, and it is very questionable, therefore, whether, with moderate doses of these substances, enough would remain unabsorbed at the lower end of the small intestine to exert much influence upon the growth and development of micro-organisms. Certainly, the fæces do not ordinarily contain any appreciable amount of borax or boric acid after these substances have been administered in moderate quantities, although obviously the length of time the fæces are forming will have some influence upon their content of soluble matter. In only one instance, to be detailed later, where a very large dose of borax was given, could any decided reaction for boric acid be obtained in the fæces. Johnson[*] states that in the case of the human organism borax and boric acid show great irregularity in their appearance in the fæces, and that he was able to detect them in the latter only in six cases out of fourteen, although daily doses of 0.9–3.0 grams of boric acid were given.

Lastly, it is to be noted that in our experiment with boric acid there is no such increase in the excretion of phosphoric

---

[*] Johnson, Ueber die Ausscheidung von Borsäure und Borax aus dem menschlichen Organismus. Jahresbericht f. Thierchemie, 1885, p. 235.

acid through the urine as was observed by Forster; our results, indeed, fail to show any distinct influence exerted by boric acid upon the metabolism of phosphorized matter.

Third Experiment. With Borax and Boric Acid. — This experiment was divided into seven periods of eight days each, thus making a total of fifty-six consecutive days during which the variations in the composition of the urine and fæces were followed as before, under the influence of both borax and boric acid. The object in extending the experiment through this lengthy period was to ascertain whether prolonged treatment with borax and boric acid might not eventually result in such a disturbance of physiological equilibrium that more positive data would be obtained. With this end in view, a mongrel bitch of ten kilos body-weight was brought into nitrogenous equilibrium, after which the urine and fæces were analyzed for eight consecutive days, *i. e.*, the fore period. Borax was then given with the food for eight days, making the first borax period. This was followed by another period of eight days during which neither borax nor boric acid were administered, after which came a third period of eight days when boric acid was fed. This, in turn, was succeeded by a normal period of equal length, followed by eight days of borax treatment — the second borax period — concluding with a final after period of eight days, *i. e.*, a total of fifty-six days. By thus keeping the same animal under continuous observation for this length of time it might reasonably be expected that any cumulative action — assuming it to exist — would be clearly manifest. Further, considerably larger daily doses of borax and boric acid were administered than in the preceding experiments.

The daily diet made use of throughout the entire experiment consisted of 160 grams of the prepared meat, 40 grams of cracker dust, 30 grams of lard, and 430 c.c. of water. Its exact content of nitrogen is shown in the table of the fore period. The total amount of nitrogen ingested during the fore period was 52.163 grams. The amount excreted during the same period was 51.734 grams, thus showing a nitrogen

balance for the eight normal days of + 0.429 grams. The dog used in this experiment, although short-haired, lost considerable hair daily. This was therefore collected and at the end of each period its content of nitrogen was determined and the amount added to the nitrogen of the urine and fæces, as seen in the accompanying tables. It is interesting to note in this connection that the loss of hair in periods of eight days' duration may be considerable; so large, indeed, that an appreciable loss of nitrogen may result. Thus, in the seven periods of this experiment the total amount of hair shed was 61.98 grams, *i. e.*, 8–10 grams for each period, the total nitrogen thrown off in this manner amounting to 7.856 grams. These figures show that the hair shed contained 12.6 per cent of nitrogen. Obviously, in careful experiments, this source of loss cannot be overlooked.

In the first borax period of eight days, the daily dose of borax ranged from 2 to 5 grams, the total amount administered being 32.5 grams. In the following boric acid period the daily dose ranged from 1 to 3 grams, a total of 17 grams of boric acid being given. On commencing the second borax period the daily dose of borax was placed at 10 grams. This was continued for two days, but on the third day after taking the morning dose of 5 grams the animal's appetite began to fail so that it became necessary to coax her considerably in order to have the day's ration consumed. On this day, therefore, only 5 grams were given, but on the following day the appetite was nearly normal and 6 grams of borax were given. The dose was then raised to 10 and 8 grams daily, as shown in the tables, a total of 64 grams of borax being given in this period of eight days. Throughout the entire experiment of fifty-six days the animal remained perfectly well, kept a fairly constant body-weight, and showed no symptoms of nausea or vomiting during the administration of either borax or boric acid. The only noticeable effect was a seeming loss of appetite on one day, as mentioned above. At the termination of the final after period, a single dose of 5 grams of boric acid was given. This resulted in vomiting 4–5 hours afterward.

THIRD EXPERIMENT.—FORE PERIOD.

| Date. | Body. | Food. | Urine. | | | | | | | | Fæces. | |
1897.	Weight.	Nitrogen.	Volume.	Sp. gr.	Reaction (litmus).	Nitrogen.	Uric Acid.	Total SO₃.	Combined SO₃.	Total P₂O₅.	Dry weight.	Nitrogen.
	kilos.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.
April 21	10.0	6.593	490	1015	Acid.	6.160	0.040	0.525	0.042	0.981	:	:
22	10.0	6.770	470	1013	Acid.	5.050	0.032	0.487	0.036	0.697	:	:
23	10.0	6.770	540	1016	Acid.	7.189	0.042	0.641	0.071	1.117	:	:
24	10.1	6.406	440	1014	Acid.	5.231	0.028	0.489	0.059	0.779	:	:
25	10.0	6.406	640	1015	Acid.	7.685	0.060	0.746	0.098	1.329	14.33	0.846
26	10.0	6.406	465	1012	Acid.	4.643	0.031	0.420	0.039	0.638	:	:
27	10.0	6.406	525	1014	Acid.	5.641	0.047	0.544	0.061	0.862	:	:
28	9.9	6.406	626	1015	Acid.	7.544	0.085	0.713	0.094	1.335	10.36	0.571
					Nitrogen of Urine = 49.083 Nitrogen of Fæces = 1.417 Nitrogen of Hair = 1.224							
Totals · · ·		52.163	4196			51.784	0.315	4.515	0.495	7.788	24.69	1.417
Daily Averages		6.520	525			6.467	0.039	0.564	0.062	0.967		0.177

THIRD EXPERIMENT.—FIRST BORAX PERIOD.

Date. 1897.	Body. Weight.	Food. Nitrogen.	Borax.	Urine.								Faeces.	
				Vol.	Sp. gr.	Reaction (litmus).	Nitrogen.	Uric Acid.	Total SO_3.	Combined SO_3.	Total P_2O_5.	Dry Weight.	Nitrogen.
	kilos.	grm.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.
April 29	10.0	6.406	2	400	1015	Alkaline.	4.025	0.039	0.401	0.049	0.597	2.96	0.168
30	10.1	6.406	3	400	1022	Alkaline.	6.738	0.043	0.677	0.091	1.142	⋮	⋮
May 1	10.0	6.406	4	591	1018	Alkaline.	6.542	0.042	0.704	0.089	1.107	⋮	⋮
2	10.0	6.406	4	470	1021	Alkaline.	7.028	0.042	0.797	0.126	1.089	⋮	⋮
3	9.9	6.406	4.5	520	1017	Alkaline.	5.916	0.031	0.565	0.072	0.781	20.10	0.990
4	10.1	6.285	5	380	1017	Alkaline.	4.041	0.024	0.372	0.040	0.409	⋮	⋮
5	10.1	6.285	5	460	1022	Alkaline.	6.531	0.040	0.597	0.082	0.977	⋮	⋮
6	10.0	6.285	5	540	1022	Alkaline.	7.503	0.032	0.792	0.118	1.272	20.69	1.023
Totals	....	50.885	32.5	3761			51.686	0.293	4.905	0.682	7.374	43.75	2.176
Daily Averages		6.361	4.06	470			6.461	0.037	0.613	0.083	0.922		0.272

Nitrogen of Urine = 48.324
Nitrogen of Faeces = 2.176
Nitrogen of Hair = 1.186

THIRD EXPERIMENT.—FIRST AFTER PERIOD.

Date. 1897.	Body. Weight.	Food. Nitrogen.	Urine. Vol.	Sp. gr.	Reaction (litmus).	Nitrogen.	Uric Acid.	Total SO₃.	Combined SO₃.	Total P₂O₅.	Faeces. Dry Weight.	Nitrogen.
	kilos.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.
May 7	10.1	6.285	410	1015	Acid.	5.687	0.086	0.575	0.054	0.810	:	:
8	10.2	6.285	430	1012	Acid.	4.330	0.034	0.449	0.040	0.458	:	:
9	10.1	6.285	590	1016	Acid.	7.671	0.044	0.623	0.117	1.187	:	:
10	10.2	6.428	380	1014	Acid.	4.717	0.014	0.568	0.005	0.599	:	:
11	10.0	6.428	597	1015	Acid.	7.425	0.036	0.872	0.106	1.423	19.55	0.745
12	10.0	6.428	530	1013	Acid.	5.952	0.029	0.586	0.060	1.066	:	:
13	10.1	6.428	525	1014	Acid.	5.894	0.029	0.620	0.065	1.017	11.90	0.627
14	10.1	6.428	490	1013	Acid.	5.764	0.026	0.568	0.055	0.959	8.21	0.478
Totals	......	50.995	3962			50.334	0.248	4.856	0.652	7.519	39.66	1.845
Daily Averages		6.374	495			6.292	0.031	0.607	0.070	0.940		0.231

Nitrogen of Urine = 47.480
Nitrogen of Faeces = 1.845
Nitrogen of Hair = 1.059

THIRD EXPERIMENT.—BORIC ACID PERIOD.

Date.	Body. Weight.	Food. Nitrogen.	Boric Acid.	Vol.	Sp. gr.	Reaction (litmus).	Urine. Nitrogen.	Uric Acid.	Total SO₃.	Combined SO₃.	Total P₂O₅.	Fæces. Dry Weight.	Fæces. Nitrogen.
1897.	kilos.	grm.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.
May 15	10.1	6.428	1	525	1015	Acid.	5.677	0.089	0.558	0.068	1.003	2.73	0.157
16	10.1	6.396	1	441	1015	Acid.	5.424	0.035	0.627	0.066	0.785	...	...
17	10.2	6.396	1.5	401	1014	Acid.	4.247	0.053	0.454	0.038	0.502	...	...
18	10.2	6.396	2	490	1015	Acid.	5.909	0.018	0.637	0.076	0.927	9.90	0.529
19	10.1	6.396	2.5	555	1016	Acid.	6.984	0.081	0.784	0.100	1.184	...	...
20	10.2	6.396	3	465	1016	Acid.	6.131	0.041	0.606	0.080	0.806	9.68	0.557
21	10.2	6.396	3	400	1014	Acid.	4.588	0.034	0.457	0.042	0.467	...	...
22	10.3	6.396	3	500	1018	Acid.	7.029	0.059	0.689	0.099	1.080	11.86	0.579
Totals		51.200	17	3777			49.026	0.310	4.762	0.569	6.754	34.17	1.822
Daily averages		6.400	2.1	472			6.128	0.089	0.595	0.071	0.844		0.228

Nitrogen of Urine = 45.939
Nitrogen of Fæces = 1.822
Nitrogen of Hair = 1.265

THIRD EXPERIMENT. — SECOND AFTER PERIOD.

Date. 1897.	Body. Weight.	Food. Nitrogen.	Urine. Vol.	Sp. gr.	Reaction (litmus).	Nitrogen.	Uric Acid.	Total SO₃.	Combined SO₃.	Total P₂O₅.	Faeces. Dry Weight.	Nitrogen.
	kilos.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.
May 23	10.3	6.396	402	1015	Acid.	5.424	0.051	0.597	0.075	0.671	3.95	0.192
24	10.3	6.396	445	1010	Alkaline.	3.957	0.028	0.394	0.081	0.289	...	...
25	10.1	6.410	620	1014	Acid.	7.224	0.066	0.787	0.077	1.115	...	...
26	10.2	6.410	521	1013	Acid.	5.730	0.051	0.541	0.045	0.911	0.76	0.542
27	10.1	6.410	550	1014	Acid.	5.614	0.039	0.601	0.050	0.892	...	...
28	10.1	6.410	470	1016	Acid.	6.518	0.083	0.722	0.066	1.108	...	...
29	10.2	6.410	455	1013	Alkaline.	4.994	0.041	0.549	0.043	0.692	...	...
30	10.3	6.410	480	1017	Acid.	6.977	0.045	0.769	0.065	1.118	15.04	0.731
Totals		51.252	3943			49.130	0.354	4.960	0.452	6.786	28.75	1.465
Daily Averages		6.406	493			6.141	0.044	0.620	0.056	0.848		0.183

Nitrogen of Urine = 46.438
Nitrogen of Faeces = 1.465
Nitrogen of Hair = 1.227

THIRD EXPERIMENT.—SECOND BORAX PERIOD.

Date.	Body.	Food.	Borax.	Urine.								Fæces.	
1897.	Weight.	Nitrogen.		Vol.	Sp. gr.	Reaction (litmus).	Nitrogen.	Uric Acid.	Total SO₃.	Combined SO₃.	Total P₂O₅.	Dry Weight.	Nitrogen.
	kilos.	grm.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.
May 31	10.1	6.410	10	580	1027	Alkaline.	7.711	0.053	0.836	0.070	1.474	...	...
June 1	10.1	6.410	10	420	1029	Alkaline.	6.384	0.087	0.671	0.052	1.102	...	...
2	10.3	6.410	5	360	1026	Alkaline.	6.627	0.029	0.761	0.099	0.998	19.10	0.874
3	10.4	6.410	6	342	1020	Alkaline.	4.574	0.029	0.495	0.038	0.836	...	...
4	10.3	6.392	7	540	1022	Alkaline.	8.025	0.042	0.828	0.118	1.112	...	...
5	10.2	6.374	10	450	1025	Alkaline.	5.634	0.031	0.610	0.077	0.680	19.40	1.019
6	10.2	6.374	8	502	1023	Alkaline.	6.495	0.040	0.629	0.070	1.005	...	...
7	10.3	6.374	8	513	1028	Alkaline.	6.913	0.034	0.683	0.076	0.809	17.55	0.844
						Nitrogen of Urine = 52.363	56.082						
						Nitrogen of Fæces = 2.737							
						Nitrogen of Hair = 0.982							
Totals	...	51.154	64	3657			56.082	0.295	5.513	0.595	7.516	56.05	2.737
Daily Averages		6.394	8	457			7.004	0.037	0.689	0.074	0.939		0.342

THIRD EXPERIMENT.—THIRD AFTER PERIOD.

Date.	Body.	Food.	Urine.								Faeces.	
1897.	Weight.	Nitrogen.	Vol.	Sp. gr.	Reaction (litmus).	Nitrogen.	Uric Acid.	Total SO₃.	Combined SO₃.	Total P₂O₅.	Dry Weight.	Nitrogen.
	kilos.	grm.	c.c.			grm.	grm.	grm.	grm.	grm.	grm.	grm.
June 8	10.3	6.374	411	1016	Acid.	6.213	0.042	0.567	0.033	0.644	...	...
9	10.3	6.374	525	1013	Acid.	5.834	0.029	0.558	0.043	0.686	...	...
10	10.4	6.374	422	1011	Acid.	4.284	0.042	0.447	0.047	0.899	...	...
11	10.4	6.374	500	1014	Acid.	6.149	0.037	0.638	0.068	0.872	...	...
12	10.4	6.374	525	1016	Acid.	7.560	0.051	0.781	0.075	1.344	22.80	0.895
13	10.3	6.409	508	1013	Alkaline.	5.158	0.043	0.518	0.030	0.644	...	...
14	10.2	6.445	652	1015	Acid.	7.917	0.040	0.856	0.082	1.445	...	...
15	10.2	6.445	512	1012	Acid.	5.663	0.044	0.631	0.065	0.793	20.06	1.194
Totals		51.169	4050			51.830	0.328	4.996	0.433	6.827	42.86	2.089
Daily Averages		6.396	506			6.479	0.041	0.624	0.054	0.853		0.261

Nitrogen of Urine = 48.778
Nitrogen of Faeces = 2.089
Nitrogen of Hair = 0.963

THIRD EXPERIMENT. — GENERAL SUMMARY.

Periods.	Total Nitrogen			Urine.						Faeces.		Hair.
	Ingested.	Excreted.	Balance.	Volume.	Nitrogen.	Uric Acid.	Total SO_3.	Combined SO_3.	Total P_2O_5.	Dry Weight.	Nitrogen.	Nitrogen.
	grm.	grm.	grm.	c.c.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
Normal .	52.163	51.734	+0.429	4196	49.093	0.315	4.515	0.495	7.738	24.09	1.417	1.224
Borax . .	50.885	51.686	−0.801	3761	48.324	0.293	4.905	0.662	7.374	43.75	2.176	1.186
After . .	50.995	50.834	+0.661	3962	47.480	0.248	4.856	0.562	7.519	39.66	1.845	1.059
Boric Acid	51.200	49.026	+2.174	3777	45.939	0.310	4.762	0.569	6.754	34.17	1.822	1.265
After . .	51.252	49.130	+2.122	3943	46.488	0.354	4.960	0.452	6.786	28.75	1.465	1.227
Borax . .	51.154	56.032	−4.878	3657	52.363	0.295	5.513	0.595	7.516	56.05	2.737	0.932
After . .	51.169	51.830	−0.661	4050	48.778	0.328	4.996	0.433	6.827	42.86	2.089	0.963

The relative excretion of nitrogen for the seven periods is shown in the following table:

	I. Fore Period.	II. First Borax Period.	III. First After Period.
Nitrogen of Food . . .	52.163	50.885	50.995
Nitrogen of Urine . . .	49.093 ⎫	48.324 ⎫	47.430 ⎫
Nitrogen of Fæces . . .	1.417 ⎬ 51.734	2.176 ⎬ 51.686	1.845 ⎬ 50.334
Nitrogen of Hair . . .	1.224 ⎭	1.186 ⎭	1.059 ⎭
Nitrogen Balance . .	+0.429	—0.801	+0.661
Ratio of Urine and Hair Nitrogen to Food Nitrogen	96.4 per cent.	97.2 per cent.	95.0 per cent.

	IV. Boric Acid Period.	V. Second After Period.
Nitrogen of Food	51.200	51.252
Nitrogen of Urine	45.939 ⎫	46.438 ⎫
Nitrogen of Fæces	1.822 ⎬ 49.026	1.465 ⎬ 49.130
Nitrogen of Hair	1.265 ⎭	1.227 ⎭
Nitrogen Balance . . .	+2.174	+2.122
Ratio of Urine and Hair Nitrogen to Food Nitrogen .	92.2 per cent.	93.0 per cent.

	VI. Second Borax Period.	VII. Third After Period.
Nitrogen of Food	51.154	51.169
Nitrogen of Urine	52.363 ⎫	48.778 ⎫
Nitrogen of Fæces	2.737 ⎬ 56.032	2.089 ⎬ 51.830
Nitrogen of Hair	0.932 ⎭	0.963 ⎭
Nitrogen Balance . . .	—4.878	—0.661
Ratio of Urine and Hair Nitrogen to Food Nitrogen .	104.1 per cent.	97.2 per cent.

In the first borax period of eight days, with a total consumption of 32.5 grams of borax, *i. e.*, an average of 4 grams per day, there is practically no change in the rate of proteid metabolism. There is, however, a slight rise in the amount of fæcal nitrogen similar to that noticed in the first experiment with borax, by which the nitrogen balance is somewhat changed, but there is plainly no effect produced on proteid metabolism. In the second borax period, on the other hand, there is evidence for the first time of a distinct and unques-

tionable influence upon proteid metabolism. In this period of eight days 64 grams of borax were administered, and under its influence the excretion of nitrogen through the urine was greatly increased. As in the other experiments, the proportion of nitrogen in the fæces was likewise increased, implying decreased assimilation of proteid food, but the nitrogen balance of − 4.878 is mainly due to direct stimulation of proteid metabolism. When, however, it is considered that to accomplish this result a daily dose of 8 grams of borax was required, and for eight consecutive days, with a dog weighing only 10 kilos, it is very plain that proteid metabolism is not readily affected by borax.

In the boric acid period of eight days, with a total dosage of 17 grams of the acid, there is some evidence of diminished proteid metabolism. The excretion of nitrogen through the urine is certainly diminished; there appears to be a sparing of proteid, but it is to be noticed that, in the period following, the nitrogen balance remains unaltered, which fact casts some doubt upon the assumption that the result is due solely to the acid. It is of course possible that the action of the boric acid may be continued into the after period, but this we should hardly expect in view of the rapid elimination of boric acid from the system. Further, after the second borax period, where the nitrogen balance is so noticeably disturbed, there is a quick return to the normal, the nitrogen balance for the final period dropping back to − 0.661. Consequently, while the analytical data show a retention of nitrogen during the boric acid period, thus indicating diminished proteid metabolism, we feel some hesitation in attributing the result wholly to the boric acid, particularly as the earlier experiment with boric acid gave essentially negative results.

Especially noticeable in this experiment, as in the earlier experiment with borax, is the action of the latter agent in reducing the volume of the urine. (See table showing general summary.) In both borax periods the total volume of urine excreted is distinctly reduced, and the same holds true in this experiment with the boric acid. It is quite probable that the

somewhat larger daily dose of boric acid made use of in the
present experiment is responsible for this result, although it
is possible of course that the personality of the animal may
have had some influence.   In the previous experiment with
boric acid, where the maximum daily dose was 2 grams, the
volume of the urine was unaltered.   In view of these facts it
is perhaps proper to consider the larger dosage of boric acid
used in the present experiment as responsible for the apparent
action upon proteid metabolism likewise.

Also noticeable in this experiment is the influence of the
larger doses of borax upon the excretion of total and com-
bined sulphuric acid.   Both of these are distinctly increased
in amount during the last borax period, in harmony with the
increase in proteid metabolism, and there is a suggestion of
the same influence in the first borax period.   Moreover, in
the last borax period the excretion of phosphoric acid is
noticeably increased, while the elimination of uric acid is
slightly diminished.   It is thus plainly evident, as already
stated, that while moderate doses of borax, even long-con-
tinued, are without influence upon the nutritional processes
of the body, large doses may distinctly increase the rate of
proteid metabolism, giving rise not only to an increased
excretion of nitrogen, but also of sulphuric acid and phos-
phoric acid.

In all of these experiments with borax there is constant
evidence of an increase in the weight of the fæces during the
borax periods.   This increase in weight is due in part to an
increased output of nitrogenous matter through this channel,
but whether the latter is caused by diminished digestion and
absorption of the proteid food or to a stimulation of the
mucous or other secretions from the gastro-intestinal tract
is not so clear.   It has been plainly shown, however, in
another connection * that while borax in moderate quantities
has no inhibitory action whatever on either gastric or pan-
creatic digestion of proteids, larger proportions do retard the

* Chittenden : Influence of Borax and Boric Acid on Digestion. Dietetic
and Hygienic Gazette, 1893, vol. 9, p. 25.

proteolytic action of both digestive fluids. Further, retardation of proteolysis with borax is much more pronounced than with boric acid; hence it seems quite probable that the increased bulk of fæces and the higher content of nitrogen therein during the borax periods is due mainly to slight retardation in the assimilation of proteid food.

Large amounts of borax likewise interfere with the assimilation of fatty foods; a statement which does not appear to be true of boric acid. In the accompanying table (page 38) are given the results of our analyses of the dry fæces, from a study of which it is plain that under the influence of large doses of borax — first and second borax periods of experiment third — both the total and percentage amounts of ether-soluble matter in the fæces are greatly increased. Boric acid, on the other hand, produces no such effect. In the first experiment, with borax, the evidence of decreased fat absorption is less pronounced, although both the dosage of borax and the amount of fat fed were greater than in the first borax period of experiment third. Quite possibly this apparent difference in action may be due to the personality of the animal. However this may be, it is plain that large doses of borax are prone to increase somewhat the bulk of the fæces, in part by diminishing slightly the assimilation of both proteid and fatty food, and in part, we think, through a tendency to increase the secretion of mucus. Thus, we observed in the last experiment, during the period when the largest doses of borax were given, that the fæces were more slimy than in the normal periods, and appeared to contain more mucus than ordinarily. Further, it is to be noted that under the influence of large doses of borax there is a tendency toward diarrhœa; not very marked to be sure, but sufficient to render the discharge of fæces somewhat watery.

In spite of these evidences of minor action in the intestinal tract with large doses of borax, there is no evidence whatever of any influence exerted upon intestinal putrefaction, either by borax or boric acid. Even with the largest doses of borax the combined sulphuric acid of the urine is raised rather than

TABLE SHOWING CONTENT OF FAT AND OTHER ETHER-SOLUBLE MATTER IN THE FÆCES.

EXPERIMENT I.

Date. 1896.	Fæces. Dry weight. grm.	Ether-soluble matter. per cent.	grm.	Period.
Dec. 2	38.15	35.03	13.362	Fore
7	35.91	33.60	12.067	
11	24.68	25.23	6.227	
	60.59	30.02	18.294	Borax
16	33.25	36.51	12.140	
20	25.45	24.36	6.198	
	58.70	31.24	18.338	After

EXPERIMENT II.

Date. 1897.	Fæces. Dry weight. grm.	Ether-soluble matter. per cent.	grm.	Period.
Feb. 26	6.96	23.70	1.649	
Mar. 2	11.90	17.88	2.128	
3	10.50	16.95	1.770	
5	17.30	20.82	3.602	
	46.66	19.61	9.149	Fore
8	10.20	18.87	1.924	
10	9.75	17.67	1.723	
12	16.30	20.81	3.311	
14	11.60	20.60	2.390	
15	5.45	20.54	1.119	
	53.20	19.67	10.467	Boric Acid
16	5.45	20.54	1.119	
17	7.71	26.63	2.053	
19	8.82	20.28	1.789	
21	12.25	20.72	2.538	
23	10.47	20.01	2.095	
25	10.90	19.31	2.105	
	55.60	21.04	11.699	After

EXPERIMENT III.

Date. 1897.	Fæces. Dry weight. grm.	Ether-soluble matter. per cent.	grm.	Period.
Apr. 25	14.83	28.91	4.134	
28	10.36	29.09	3.029	
	24.69	29.01	7.163	Normal
29	2.96	29.09	0.840	
May 3	20.10	36.35	7.306	
6	20.69	37.06	7.671	
	48.75	36.15	15.817	Borax
11	19.55	36.18	7.091	
13	11.90	23.50	2.797	
14	8.21	25.89	2.117	
	39.66	30.27	12.005	After
15	2.73	25.89	0.705	
18	9.90	33.19	3.286	
20	9.68	25.76	2.499	
22	11.86	24.13	2.858	
	34.17	27.36	9.348	Boric Acid
23	3.95	24.13	0.953	
26	9.76	24.20	2.372	
30	15.04	29.54	4.443	
	28.75	27.02	7.768	After
June 2	19.10	45.01	8.596	
5	19.40	39.06	7.579	
7	17.55	33.94	5.940	
	56.05	39.46	22.115	Borax
12	22.80	39.27	8.954	
15	20.06	29.99	6.028	
	42.86	34.96	14.982	After

lowered, and careful examination of the urine daily with Jaffe's indoxyl test failed to reveal any indications pointing to an inhibitory influence exerted by either borax or boric acid upon the production of indican. If, however, one studies carefully the output of combined sulphuric acid as shown in the various tables it will be noticed that the highest figures are generally obtained on the day (or the day preceding that) on which the dog defecates; while after defecation the combined sulphuric acid of the urine falls at once. In other words, the natural obstruction of the intestine favors, as is well known, the absorption of putrefactive products, and thus leads to an increase of combined sulphuric acid in the urine. When, on the other hand, defecation occurs, the combined sulphuric acid of the urine is at once diminished in amount. Upon these natural fluctuations of combined sulphuric acid even the largest doses of borax and boric acid are without effect, not because these agents are without influence upon micro-organisms, but because they are too rapidly and completely absorbed from the intestine to exert much influence upon intestinal putrefaction. In only one instance were we able to detect any boric acid in the fæces, viz., on June 5th, at a time when the largest doses of borax were being given; and at the close of this period the boric acid reaction could be obtained with the urine only on the first day of the after period, so rapidly was the borax passed out of the body.

Lastly, attention may be called to the constant presence, in appreciable amounts, of uric acid in the urine of all the animals experimented with, in opposition to the older statements of Liebig * and others that kynurenic acid may entirely replace uric acid in the urine of the dog. Our results, so far as they extend, are thus wholly in accord with the recent observations of Solomin.† We have, however, made no attempt to determine the amounts of kynurenic acid present.

General Conclusions. — Moderate doses of borax up to 5 grams per day, even when continued for some time, are with-

---

* Liebig, Annalen d. Chem. u. Pharm., Band 86, p. 125.

† Solomin, Zur Kenntniss der Kynurensäure. Zeitschr. f. physiol. Chem., 1897, Band 23, p. 497.

out influence upon proteid metabolism.   Neither do they exert any specific influence upon the general nutritional changes of the body.   Under no circumstances, so far as we have been able to ascertain, does borax tend to increase body-weight or to protect the proteid matter of the tissues.

Large doses of borax, 5–10 grams daily, have a direct stimulating effect upon proteid metabolism, as claimed by Gruber; such doses, especially if continued, lead to an increased excretion of nitrogen through the urine, also of sulphuric acid and phosphoric acid.

Boric acid, on the other hand, in doses up to 3 grams per day, is practically without influence upon proteid metabolism and upon the general nutrition of the body.

Borax, when taken in large doses, tends to retard somewhat the assimilation of proteid and fatty foods, increasing noticeably the weight of the fæces and their content of nitrogen and fat.   With very large doses there is a tendency toward diarrhœa and an increased excretion of mucus.   Boric acid, on the contrary, in doses up to 3 grams per day, is wholly without influence in these directions.

Borax causes a decrease in the volume of the urine, changes the reaction of the fluid to alkaline, and raises the specific gravity, owing to the rapid elimination of the borax through this channel.   Under no circumstances have we observed any diuretic action with either borax or boric acid.   The latter agent has little effect on the volume of the urine.

Both borax and boric acid are quickly eliminated from the body through the urine, twenty-four to thirty-six hours being generally sufficient for their complete removal.   Rarely are they found in the fæces.

Neither borax nor boric acid have any influence upon the putrefactive processes of the intestine as measured by the amount of combined sulphuric acid in the urine, or by Jaffe's indoxyl test.   Exceedingly large doses of borax are inactive in this direction, not because the salt is without action upon micro-organisms, but because of its rapid absorption from the intestinal tract.

Borax and boric acid, when given in quantities equal to 1.5–2.0 per cent of the daily food are liable to produce nausea and vomiting.

. Owing to the rapid elimination of both borax and boric acid, no marked cumulative action can result from their daily ingestion in moderate quantities.

At no time in these experiments was there any indication of abnormality in the urine; albumin and sugar were never present.

THE MUCIN OF WHITE FIBROUS CONNECTIVE TISSUE.[*]

BY R. H. CHITTENDEN AND WILLIAM J. GIES.

ALL of the bodies belonging to the group of mucins and mucoids are possessed of considerable physiological interest, owing especially to their peculiar compound nature and the illustration which they afford of a possible intimate union between the proteid group and carbohydrate radicles. That there are a number, possibly a large number, of closely related bodies belonging to the mucins and mucoids there can be no question. Thanks to the labors of Hammarsten [†] and his pupils, many of these bodies have been subjected to careful and thorough investigation, and much light has been thrown upon their relationships and differences. There is still, however, much to be ascertained regarding these bodies, and any additional facts broadening or substantiating our present knowledge are to be welcomed as contributing toward a more complete understanding of their genetic relationships. The union of carbohydrate groups with proteid molecules is probably more common than has hitherto been supposed, as witness the peculiar gluco-nucleoproteid recently described by Hammarsten [‡] as a constituent of the pancreas and other glands, and the identification by Kossel [§] of a peculiar carbohydrate group as a cleavage product of certain forms of nucleic acid. Presumably in these compound proteids of the mucin type the character of the proteid radicle as well as of

---

the carbohydrate radicle is subject to variation, and it is easy to conceive of differences in the nature and properties of the mucins dependent upon variations in the amount and character of both the carbohydrate and proteid groups. The ready formation of acid-albumin, or syntonin, albumoses, and peptone when mucins are decomposed by the action of superheated water or boiling dilute acids, affords ample evidence of the presence of true proteid radicles in the bodies of this class, although we do not know definitely the exact nature of the proteid groups present in the original molecule. On the other hand, the simultaneous formation of reducing bodies whenever mucins are broken down by the action of dilute acids, and the separation of a dextrin-like body (the animal gum of Land-wehr *) by cleavage with superheated water, clearly indicate the existence of some form of carbohydrate matter in the mucin molecule.

Of the true mucins present in the tissues of the higher animals, the mucin of the submaxillary gland and the corresponding body present in or between the fibres of ordinary connective tissue are the most important from a physiological standpoint. The former is a product of the metabolic activity of secretory cells which are among the most active of the secreting cells of the body, while the latter is a product of a tissue whose activity is certainly of a low order. That these two mucins, though closely related, are unlike, is clearly indicated by their divergence in chemical composition as well as by their general reactions and properties.

Loebisch,† whose careful study of the mucin from tendons constitutes the chief source of our knowledge regarding the chemical composition of this body, ascribes to tendon-mucin the formula $C_{160}H_{256}N_{32}S_1O_{80}$, with a molecular weight of 3,936. Such a formula calls for the presence of 0.81 per cent of sulphur, and this amount was found by Loebisch in the three preparations of mucin from ox tendons analyzed by him. In

---

* Zeitschr. f. physiol. Chem., Band viii and ix. Also Pflüger's Archiv f. Physiol., Band xxxix and xl.

† Zeitschr. f. physiol. Chem., Band x, p. 40.

a recent examination of mucin prepared from this same source we have obtained quite different results as regards the content of sulphur, and this fact has led us to make a careful study of the composition of this form of connective-tissue mucin. Our results in the main have afforded a close substantiation of the conclusions arrived at by Loebisch, with the single exception of the sulphur for which we can find no adequate explanation. Further, some additional facts have been found which are perhaps worthy of note.

The first sample of mucin studied was prepared from the Achilles tendons of oxen by the following method, analogous to the method described by Loebisch: The fresh tendons were freed as carefully as possible from all adherent tissues, then cut into very thin traverse sections with a razor, washed thoroughly with distilled water, frequently renewed for twenty-four hours, in order to remove all blood and soluble albuminous matter, and finally pressed as dry as possible. The resultant material weighed 1200 grams. In order to extract the mucin, the tissue was placed in 2.4 liters of half-saturated lime-water, where it was allowed to remain for forty-eight hours with frequent agitation. At the end of this period the pale-yellowish fluid was strained through a cloth filter and finally filtered through paper. The clear fluid was then treated with an excess of 0.2 per cent hydrochloric acid — a little more than a liter — by which a heavy flocculent precipitate resulted, quickly settling to the bottom of the cylinder, leaving a nearly clear supernatant fluid.

The residue of tendon-tissue was again extracted for forty-eight hours with 2.4 liters of half-saturated lime-water, and the resultant solution precipitated with an excess of 0.2 per cent hydrochloric acid. The precipitate so formed was nearly as heavy as the first, thus showing that extraction of the mucin by weak lime-water is a slow and gradual process.

The precipitated mucin, separated from the acid fluid by subsidence and decantation of the supernatant liquid, was washed thoroughly with 0.2 per cent hydrochloric acid, whipping up the precipitate with the fluid and then allowing it to

subside, this operation being repeated with fresh quantities of acid until the latter failed to give any proteid reaction. In this manner it was hoped to remove all adherent albuminous matter extracted from the tissue by the lime-water. The two portions of mucin were then united and washed by decantation with distilled water until the acid was entirely removed. As the fluid became less and less acid, more time was required for the precipitate to settle, as the latter tended to swell in the water and was more inclined to float on the surface of the fluid.

The mucin was next dissolved in half-saturated lime-water, of which a large volume was required, the solution filtered through paper, and the mucin reprecipitated by the addition of an excess of 0.2 per cent hydrochloric acid, a small quantity of stronger hydrochloric acid being likewise added to induce a good flocculent separation of the substance. The precipitate was again washed by decantation with 0.2 per cent hydrochloric acid, and lastly with water, until the acid was entirely removed. Whenever it was necessary for the precipitate to stand for some time with water, the mixture was kept as cool as possible, and a little alcoholic solution of thymol added to guard against putrefactive changes. When the acid was wholly removed from the precipitate the water was replaced by weak alcohol, and finally by 95 per cent alcohol, repeatedly renewed, until the substance was thoroughly dehydrated, after which the precipitate was collected on a filter and allowed to drain. It was then boiled with alcohol-ether (a mixture of equal parts absolute alcohol and ether) in a suitable flask connected with an inverted Liebig's condenser for many days — *i. e.*, with renewed quantities of alcohol-ether until the latter gave no residue on evaporation. As Loebisch has shown, this is quite an important part of the process of purification, since a certain amount of foreign extractive matter adheres tenaciously to the mucin, and can be removed only by long-continued extraction with the above mixture. When this process was completed the mucin was thrown upon a filter, washed thoroughly with ether, and finally dried over sulphuric acid. When quite dry it presented the appearance of a perfectly white powder, light

and fluffy. The yield amounted to twelve grams of the dry product, and, assuming that the entire amount of mucin had been extracted from the tendons, and disregarding the loss incidental to purification, this quantity would imply the presence in the fresh tendons of 1 per cent of mucin.

The composition of the product, dried at 110° C. until of constant weight, was as follows: *

Preparation No. 1.

I. 0.2670 gram of substance gave 0.4781 gram of $CO_2 = 48.84$ per cent C, and 0.1585 gram of $H_2O = 6.60$ per cent H.

II. 0.2277 gram of substance gave 0.4082 gram of $CO_2 = 48.89$ per cent C, and 0.1329 gram of $H_2O = 6.48$ per cent H.

III. 0.1975 gram of substance gave 0.3548 gram of $CO_2 = 48.99$ per cent C.

IV. 0.2363 gram of substance gave 0.1417 gram of $H_2O = 6.66$ per cent H.

V. 0.2426 gram of substance gave, by the Kjeldahl method, 0.02865 gram of nitrogen $= 11.81$ per cent N.

VI. 0.2754 gram of substance gave, by the Kjeldahl method, 0.03246 gram of nitrogen $= 11.79$ per cent N.

VII. 0.2784 gram of substance gave, by the absolute method, 27.63 c. c. of nitrogen at 13.3° C., and 764.7 mm. pressure $= 11.96$ per cent N.

VIII. 0.3345 gram of substance gave, by the absolute method, 33.3 c. c. of nitrogen at 13.2° C., and 754.5 mm. pressure $= 11.84$ per cent N.

IX. 0.5373 gram of substance gave, by fusion with $NaOH + KNO_3$, 0.0943 gram of $BaSO_4 = 2.41$ per cent S; after deducting sulphur of ash $= 2.36$ per cent S.

X. 0.4969 gram of substance gave, by fusion with $NaOH + KNO_3$, 0.0856 gram of $BaSO_4 = 2.37$ per cent S; after deducting sulphur of ash $= 2.32$ per cent S.

XI. 0.2943 gram of substance gave 0.0023 gram of ash $= 0.78$ per cent ash.

XII. Ash from 0.2943 gram of substance gave 0.00112 gram of $BaSO_4 = 0.05$ per cent S.

Percentage Composition of the Ash-free Substance.

											Average.
C	49.22	49.27	49.37	...	...	...	...	...	...	...	49.29
H	6.65	6.54	...	6.71	...	...	...	...	...	...	6.63
N	...	...	...	...	11.90	11.88	12.05	11.93	...	...	11.94
S	...	...	...	...	...	...	...	...	2.36	2.32	2.34
O	...	...	...	...	...	...	...	...	...	...	29.80
											100.00

* The nitrogen was determined by both the absolute and the Kjeldahl method, while carbon and hydrogen were determined by combustion in oxygen gas in an open tube, the products of combustion passing over a layer of cupric oxide, chromate of lead, and metallic copper.

The second preparation of mucin was made in a somewhat different manner. The fresh tendons, freed as far as possible from foreign tissue, were cut into thin transverse sections, washed with water somewhat, then soaked for thirty-six hours in about four liters of 10 per cent salt solution, with vigorous agitation from time to time, after which the saline solution was decanted and the tissue washed with water until the chloride was entirely removed. The salt solution on dilution with water gave a distinct turbidity indicating the presence of a globulin. The application of heat likewise produced a precipitate, as did also the addition of dilute acetic and hydrochloric acids. It is thus evident that the salt solution removes at the outset quite an appreciable amount of proteid matter, with perhaps some mucin. The moist tissue, pressed as dry as possible, weighed 1700 grams. It was then extracted with 3.4 liters of half-saturated lime-water for forty-eight hours, two such extractions being made. From these extracts the mucin was precipitated by the addition of 0.2 per cent hydrochloric acid, the second extract apparently yielding as heavy a precipitate as the first. The combined precipitates were washed repeatedly by decantation with 0.2 per cent hydrochloric acid, lastly with water. The mucin was next dissolved in a little 0.5 per cent sodium carbonate, the solution filtered, made nearly neutral by the addition of a little 10 per cent hydrochloric acid, so as to avoid undue dilution, and then precipitated by 0.2 per cent hydrochloric acid. The precipitate was again washed thoroughly with 0.2 per cent hydrochloric acid, and lastly with water, until the acid was entirely removed. It was then transferred to 95 per cent alcohol, frequently renewed, and finally boiled with alcohol-ether as long as anything could be extracted. Dried over sulphuric acid, the product came out quite white, but not so bulky as the preceding preparation, and weighed a little over fifteen grams — an amount equal to about 0.9 per cent of the moist tissue.

As already stated, mucin is not readily extracted from tendons by lime-water; at least four cubic centimeters of

half-saturated lime-water are required for every gram of tissue in order to insure a complete extraction. Thus, after the second extraction of the above 1700 grams of tissue, a third extraction was made, using again three liters of half-saturated lime-water. This solution, on treatment with hydrochloric acid, gave a precipitate weighing one to two grams when purified, but it was noticeable that more acid was required in order to effect a good flocculent separation of the mucin. Even with a fourth extraction of the tissue a little mucin was obtained, showing as a decided turbidity when the alkaline fluid was made distinctly acid, but it was not until four or five days' standing that a distinct precipitate settled out even on the addition of stronger hydrochloric acid. The amount so obtained, however, was very small.

The composition of the main product obtained from the 1700 grams of tissue when dried at 110° C., until of constant weight, was as follows:

Preparation No. 2.

I. 0.3194 gram of substance gave 0.5659 gram of $CO_2 = 48.32$ per cent C, and 0.1815 gram of $H_2O = 6.31$ per cent H.

II. 0.4197 gram of substance gave 0.7471 gram of $CO_2 = 48.54$ per cent C, and 0.2446 gram of $H_2O = 6.47$ per cent H.

III. 0.4051 gram of substance gave 0.7189 gram of $CO_2 = 48.39$ per cent C, and 0.2353 gram of $H_2O = 6.45$ per cent H.

IV. 0.2519 gram of substance gave, by the Kjeldahl method, 0.02965 gram of nitrogen = 11.77 per cent N.

V. 0.2578 gram of substance gave, by the Kjeldahl method, 0.03026 gram of nitrogen = 11.74 per cent N.

VI. 0.2954 gram of substance gave, by the Kjeldahl method, 0.03446 gram of nitrogen = 11.67 per cent N.

VII. 0.6610 gram of substance gave, by fusion with $NaOH + KNO_3$, 0.1131 gram of $BaSO_4 = 2.35$ per cent S; after deducting sulphur of ash = 2.32 per cent S.

VIII. 0.5248 gram of substance gave, by fusion with $NaOH + KNO_3$, 0.0936 gram of $BaSO_4 = 2.45$ per cent S; after deducting sulphur of ash = 2.42 per cent S.

IX. 0.6724 gram of substance gave, by fusion with $NaOH + KNO_3$, 0.1140 gram of $BaSO_4 = 2.33$ per cent S; after deducting sulphur of ash = 2.30 per cent S.

X. 0.3735 gram of substance gave 0.0025 gram of ash = 0.67 per cent ash.

XI. Ash from 0.3735 gram of substance gave 0.00082 gram of $BaSO_4 = 0.03$ per cent S.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

										Average.
C	48.64	48.87	48.72	...	...	...	...	...	...	48.74
H	6.36	6.52	6.50	...	...	...	...	...	...	6.46
N	...	...	...	11.85	11.82	11.74	...	...	...	11.80
S	...	...	...	...	...	...	2.32	2.42	2.30	2.35
O	...	...	...	...	...	...	...	...	...	30.65
										100.00

A third specimen of mucin was prepared as follows: Fifteen hundred grams of ox tendons were finely divided, the tissue extracted for twenty-four hours with three liters of 10 per cent salt solution, and then with water until the salt was wholly removed. The tissue was next extracted for sixty hours with three liters of half-saturated lime-water. From this solution the mucin could be only partially separated by the addition of 0.2 per cent hydrochloric acid, quite a quantity of 10 per cent acid being required to effect a flocculent precipitation of the substance. This was purified by itself and not subjected to analysis. The tendons were again extracted with three liters of half-saturated lime-water for forty-eight hours, and from this solution the mucin was separated as a flocculent precipitate by the addition of 0.2 per cent hydrochloric acid. This precipitate was purified by washing with 0.2 per cent hydrochloric acid, solution in 0.5 per cent sodium carbonate, reprecipitation with 0.2 per cent hydrochloric acid, etc., as described under the last preparation. The yield of dry product from this second extraction of the tissue with lime-water amounted to 6.5 grams. Dried at 110° C. until of constant weight, this preparation gave the following results on analysis:

PREPARATION No. 3.

I. 0.3598 gram of substance gave 0.6292 gram of $CO_2 = 47.69$ per cent C, and 0.2072 gram of $H_2O = 6.40$ per cent H.

II. 0.2939 gram of substance gave 0.5150 gram of $CO_2 = 47.79$ per cent C, and 0.1725 gram $H_2O = 6.52$ per cent H.

III. 0.3154 gram of substance gave 0.5536 gram of $CO_2 = 47.87$ per cent C.

IV. 0.1644 gram of substance gave 0.0944 gram of $H_2O = 6.38$ per cent H.

V. 0.1965 gram of substance gave, by the Kjeldahl method, 0.02255 gram of nitrogen $= 11.47$ per cent N.

VI. 0.2495 gram of substance gave, by the Kjeldahl method, 0.02825 gram of nitrogen = 11.32 per cent N.

VII. 0.2574 gram of substance gave, by the Kjeldahl method, 0.02930 gram of nitrogen = 11.38 per cent N.

VIII. 0.6046 gram of substance gave, by fusion with $NaOH + KNO_3$, 0.1045 gram of $BaSO_4$ = 2.38 per cent S; after deducting sulphur of ash = 2.31 per cent S.

IX. 0.5408 gram of substance gave, by fusion with $NaOH + KNO_3$, 0.0931 gram of $BaSO_4$ = 2.37 per cent S; after deducting sulphur of ash = 2.30 per cent S.

X. 0.3128 gram of substance gave 0.0031 gram of ash = 0.99 per cent ash.

XI. Ash from 0.3128 gram of substance gave 0.00152 gram of $BaSO_4$ = 0.07 per cent S.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

										Average
C	48.17	48.26	48.34	...	...	...	...	...	...	48.26
H	6.46	6.59	...	6.44	...	...	...	...	...	6.49
N	...	...	...	...	11.59	11.43	11.50	...	...	11.51
S	...	...	...	...	...	...	...	2.31	2.30	2.31
O	...	...	...	...	...	...	...	...	...	31.43
										100.00

A comparison of the composition of these three preparations of mucin with each other, and with the mucin analyzed by Loebisch and by Hammarsten, brings out certain points of interest which merit attention:

	Mucin from Tendons.				Snail Mucin. Hammarsten.	Submaxillary Mucin. Hammarsten.
	Preparation 1.	Preparation 2.	Preparation 3.	Loebisch's average.		
C	49.29	48.74	48.26	48.30	50.32	48.84
H	6.63	6.46	6.49	6.44	6.84	6.80
N	11.94	11.80	11.51	11.75	13.65	12.32
S	2.34	2.35	2.31	0.81	1.75	0.84
O	29.80	30.65	31.43	32.70	27.44	31.20

Loebisch analyzed three distinct preparations of mucin from ox tendons, in which the carbon, hydrogen, and sulphur showed practically no variation. The nitrogen, however, varied from 11.59 to 11.84 per cent. The average content of nitrogen in his three preparations was 11.75 per cent, identical with the average of our three preparations. It is to

be noticed, however, that the carbon of our preparations shows decided variation, and it is also to be observed that a diminution in the percentage of carbon is attended in each case with a diminution in nitrogen. We may suppose that Preparation No. 3 is the purest of our products, and it is seen to agree most closely with the results obtained by Loebisch, except in the content of sulphur. The mucin from the submaxillary gland, as well as the snail mucin, are both characterized by a comparatively high content of nitrogen, while the latter product also shows a higher percentage of carbon.

Our results seemingly justify the assumption that white fibrous connective tissue contains more than one mucin, or else that the mucin obtainable from this tissue is prone to carry with it a certain amount of some other form of proteid matter which the ordinary methods of purification are not wholly adequate to remove. Our experience leads us to the belief that the surest way of obtaining a pure mucin from tendons, or at least one with a low content of carbon and nitrogen, is to first extract the finely divided tissue with 10 per cent salt solution, then after removal of the salt with water to extract the tissue with half-saturated lime-water in the proportion of two cubic centimeters for every gram of moist tissue for about twenty-four hours at ordinary room temperature. This extract may be rejected, as it is very liable to yield a mucin with a higher content of nitrogen and carbon. By extracting the tissue a second time with lime-water a mucin may be obtained with a lower content of carbon and nitrogen, as in our third preparation. It is purely an assumption, however, to say that this body with its lower percentage of carbon and nitrogen is *pure* mucin. There is at the present time no standard of purity with regard to this body, and it is quite as probable that fibrous connective tissue contains two or more mucins as that there is only one mucin in the tissue, and that any deviation from the figures obtained by Loebisch or by us in Preparation No. 3 is due to the presence of a larger or smaller amount of proteid impurity.

Undoubtedly, preliminary extraction of the tissue with salt solution tends to remove a certain amount of proteid matter, especially globulins, which might otherwise render the product impure, and possibly this is in part the cause of the lower content of carbon and nitrogen in Preparation No. 2 as contrasted with Preparation No. 1. Still there is no certainty on this point, for it is to be remembered that precipitation of the mucin requires the addition of considerable hydrochloric acid beyond neutralization of the alkaline fluid, and this excess of acid would naturally exert a marked solvent action upon any albuminous matter present. That the first lime-water extract is liable to yield a mucin with a higher content of both carbon and nitrogen the results fully indicate, and as a direct illustration of the difference in the content of nitrogen in mucin obtained from a first and second extract, we may instance the following experiment: Fifteen hundred grams of tendons finely divided, as usual, were extracted with 10 per cent salt solution for two days, then washed with water and placed in three liters of half-saturated lime-water for forty-eight hours. This first extract was then strained off, and the tissue treated a second time with a like volume of half-saturated lime-water, thus giving a second extract. From the first extract, the mucin was precipitated by hydrochloric acid slightly above 0.2 per cent, the precipitate washed with 0.2 per cent hydrochloric acid, then with water, and lastly dissolved in 0.5 per cent sodium carbonate. From this filtered solution a portion of the mucin was precipitated by addition of 0.2 per cent hydrochloric acid, while a second portion separated only on addition of a somewhat increased strength of acid. These two fractions were washed thoroughly with 0.2 per cent acid, then with water, and finally boiled with alcohol-ether until quite free from soluble matter. The yield in the first fraction was 1.4 grams, and in the second fraction 1.0 gram. From the second lime-water extract the mucin was precipitated with 0.2 per cent hydrochloric acid, after which it was purified by washing with 0.2 per cent acid, solution in 0.5 per cent sodium carbonate,

reprecipitation with 0.2 per cent acid, etc. The content of nitrogen in the three products, when dried at 110° C., was as follows, calculated on the ash-free substance

First Extract.		Second Extract.
First fraction. 12.26 N.	Second fraction. 11.91 N.	11.51 N.

It is thus seen that the first extraction with lime-water furnishes a mucin with a considerably higher percentage of nitrogen than the second extract. It is equally noticeable that the mucin first precipitated — as in the first fraction of the first extract — has a higher percentage of nitrogen than the second fraction, thus indicating that the higher content of nitrogen and probably of carbon also belongs to some body more readily precipitated by acid than the mucin with 11.51 per cent of nitrogen. In view of the great care exercised in all of these preparations, and the ready solubility of ordinary forms of albuminous matter in an excess of hydrochloric acid, especially after they have once been dissolved in an alkaline fluid, we are very much inclined to believe in the existence of several related mucins as components of ordinary white fibrillar connective tissue.

Such a view presents no great difficulty. Submaxillary mucin, for example, differs from tendon mucin by only 0.5 per cent of carbon (48.84 per cent) and about 0.5 per cent of nitrogen (12.32 per cent), although it shows some other points of difference, such as a tendency to undergo alteration by the action of lime-water and by being soluble in 0.2 per cent hydrochloric acid. Indeed, all of the various mucins described show minor points of difference, although agreeing in their general reactions, and it is easy to conceive of the presence of two or more closely related mucins, in tendons, with different elementary composition.

The most remarkable thing, however, connected with the mucins that we have separated from this form of fibrillar connective tissue is the amount of sulphur present in the purified products. In snail mucin, Hammarsten has shown

the presence of 1.75 per cent of sulphur, but in the mucin from the submaxillary gland and in the mucin described by Loebisch as contained in tendons, the amount of sulphur has been placed at 0.84 to 0.81 per cent. In all three of our preparations, however, the sulphur present has amounted to at least 2.30 per cent, and, moreover, the agreement in the several products has been very close indeed. The greater portion of this sulphur is closely combined, a small amount only being in the form of the mercaptan group and responding to the reaction with potassium hydroxide and plumbic acetate. We present these figures with some doubt in our own minds, but, having obtained them as the result of most careful work, we see no possible explanation other than that this amount of sulphur is actually present in the mucin molecule. The determinations of sulphur were made after the usual method recommended by Hammarsten — viz., oxidizing the mucin with a mixture of ten grams NaOH and two grams KNO_3 in a silver crucible, etc. The sodium hydroxide employed was chemically pure, having been prepared from the metal, and, furthermore, several blank tests were made to prove the freedom of the various chemicals from sulphur. This percentage of sulphur is greater than has ever been accredited to a true mucin, although the mucin from the snail's membrane (mantle-mucin), which is somewhat related to keratin, has been found by Hammarsten to contain a fairly large amount of this element — viz., 1.79 per cent.

With regard to the *reactions* of the several products that we have studied, there is nothing special to be said. They all show the ordinary reactions of mucin as described by Loebisch, and we can simply substantiate what has long been published by him upon this point.

The most characteristic feature of mucin is the peculiar cleavage it undergoes when heated with dilute hydrochloric acid, by which a substance with reducing action upon alkaline copper solution results. Albumose and peptone are likewise formed by the action of the hot acid. We have tried several

preliminary experiments in this direction, the results of which may be briefly stated: 3.25 grams of mucin of Preparation No. 2 were heated in a boiling water-bath with one hundred cubic centimeters of 2 per cent hydrochloric acid for five hours. At the end of this period the solution was of a deep-brown color, while suspended through the fluid was a large amount of gelatinous matter more or less brown in color. This was filtered off, washed with water, in which it was wholly insoluble, until the washings gave no proteid reaction. It was then tested, with the following results: it was insoluble in dilute and stronger hydrochloric acid, but readily soluble in 0.5 per cent sodium carbonate and in very dilute (0.5 per cent) potassium hydroxide. From the solution in sodium carbonate it was reprecipitated by neutralization, and was then readily soluble in a slight excess of 0.2 per cent hydrochloric acid. It gave the ordinary color reactions characteristic of proteid matter. Warmed at 40° C. with an active gastric juice containing 0.2 per cent hydrochloric acid, it was wholly unaffected even after twenty-four hours, but when warmed with an alkaline pancreatic juice it was readily dissolved, and almost completely converted into products soluble even on neutralization of the fluid, thus attesting its conversion into soluble albumoses and peptones. These reactions suggest that the substance in question is a form of antialbumid.

The original acid fluid containing the soluble products formed in the cleavage of the mucin was made neutral, by which a slight neutralization precipitate resulted, evidently syntonin from the reactions tried. The neutral fluid was then concentrated to a syrup, a strong caramel-like odor being developed during the process, and while still warm the residue was treated with a large excess of 95 per cent alcohol, by which a thick gummy mass was formed, hard and brittle on cooling. While warm, the alcoholic fluid was quite clear and yellowish-red in color, but on cooling, a light-yellow precipitate, very small in quantity, formed, which was soluble in water, and gave a strong reducing action with Fehling's solution. It was too small in quantity, however, to study further.

The gummy precipitate was washed by warming it repeatedly with fresh quantities of alcohol. It was readily soluble in water, gave more or less of a proteid reaction, and showed a fairly strong reducing action with Fehling's solution. Tested with phenylhydrazine hydrochloride, and sodium acetate, only an amorphous precipitate resulted from which a crystalline osazone could not be obtained. On boiling the gummy mass with 2 per cent hydrochloric acid, however, and then extracting the neutralized and evaporated fluid with alcohol, a very small amount of a crystalline osazone was obtained by application of the hydrazine test, apparently identical with that described further on.

The original alcoholic solution from the above gummy precipitate was evaporated to a small bulk on the water-bath, the residue taken up with fifteen cubic centimeters of water forming a clear solution. This solution showed strong reducing action with alkaline copper solution, and evidently contained the greater portion of the reducing body formed from the cleavage of the mucin. To the main bulk of this solution was added one gram of phenylhydrazine hydrochloride and 1.5 grams of sodium acetate, after which the mixture was heated on the water-bath for an hour and a half, the volume of the fluid being kept at fifteen to twenty cubic centimeters. While hot the fluid was perfectly clear and reddish in color. After standing an hour in a cool place there was a marked separation of amorphous particles and oily globules, but no crystals could be detected under the microscope. After standing fifteen hours the amorphous particles were almost wholly transformed into fine crystals. These crystals were light-yellow in color, and were mostly arranged in rosettes or balls of fine yellow needles, somewhat resembling lactosazone. The oily globules were unchanged. These crystals were purified by dissolving them in cold alcohol, followed by the addition of water, and heating the solution until the alcohol was practically all removed, when the crystals again separated out as the fluid cooled. The crystals were also insoluble in the hot *concentrated* fluid. In this way the crystals were gradually

freed from the oily globules spoken of above and rendered fairly pure. Each time the crystals were filtered they were also washed with a little cold water. During the process of purification the crystals changed their appearance somewhat, tending to take on the branching form characteristic of dextrosazone. This crystalline osazone, when purified as much as possible, was readily soluble in warm water, in alcohol, ether, chloroform, and, to a certain extent, in benzol. The amount of the purified osazone was so small that the melting point alone could be determined. This was done as usual in a capillary tube. When the temperature reached 140° C. the substance commenced to darken slowly, and at 160° C. it began to melt. Further recrystallization of the osazone did not alter this melting point. In melting point, therefore, this osazone, if pure, differs widely from dextrosazone or lactosazone. In general appearance and solubility, as well as in its melting point, it appears to resemble very closely the osazone obtained by Hammarsten from the cleavage product of the peculiar nucleoproteid described by him as present in the pancreas.* Whether this body is a pentaglucose, however, we cannot definitely say. We had hoped, especially in view of the strong reducing action of the above alcoholic solution, to obtain a fairly large amount of an osazone, sufficient to determine its content of carbon and nitrogen, but the yield of purified product was very small indeed.

In order to verify the above results, a second portion of mucin was decomposed with dilute acid — 4.75 grams of mucin with two hundred and fifty cubic centimeters of 2 per cent hydrochloric acid — the mixture being heated directly over a lamp for about five hours. The flask was connected with an inverted Liebig's condenser to prevent concentration, and the mixture was kept in a state of gentle ebullition. In this case there was much less of the antialbumid-like body so prominent in the first decomposition, the amount being less than one-fifth that found before. The neutralization precipitate, however, was considerably larger, and albumose and pep-

* Zeitschr. f. physiol. Chem., Band xix, p. 19.

tone were both present in abundance. The caramel-like body precipitated by alcohol was naturally more abundant than in the first case, but on analysis it was found to contain a large percentage of nitrogen, so that its fancied resemblance to caramel is purely superficial. By evaporation of the alcoholic extract containing the greater portion of the reducing body a residue was obtained as before, from which a crystalline osazone was formed agreeing in all of its properties with the body previously described. The purified osazone melted at 158° to 160° C. It is thus evident that the mucin or mucins present in ox tendons yield on cleavage with dilute hydrochloric acid a carbohydrate body which forms a well-defined and crystalline osazone, although at present we cannot state definitely the exact nature of this carbohydrate substance.

THE GELATIN FROM WHITE FIBROUS CONNECTIVE TISSUE.*

By WILLARD G. VAN NAME.

IN a general way the chemical nature and composition of gelatin have been established for many years, but the more thorough chemical study of various forms of connective tissue, which has been carried on so successfully during the last decade, has revealed the existence of at least three distinct varieties of collagen, characterized by marked differences in chemical composition. The collagen of white fibrous tissue and of bone is generally considered as the same ; it apparently yields on hydration a gelatin with about 18 per cent of nitrogen. The collagen which makes up the greater portion of the cornea, on the other hand, contains about 17 per cent of nitrogen,† while the collagen of cartilage ‡ yields a gelatin with only 16 per cent of nitrogen. Furthermore, we have evidence of the existence of a body in reticular tissue — reticulin — the composition of which appears to lie midway between that of gelatin and of elastin.§ Its nitrogen-content, according to Siegfried, is 15.63 per cent. Again, the amount of sulphur in gelatin is usually placed at about 0.6 per cent,‖ but the gelatin formed from corneal collagen contains only 0.3 per cent of this element.¶ Lastly, Schützenberger and Burgeois consider gelatin (commercial gelatin) as practically

free from sulphur, or hold at least that this element is an unessential constituent of the molecule.*

In all of these tissues the difficulty of isolating with perfect purity the albuminoid itself is very great. In white fibrous tissue, for example, the close juxtaposition of the several anatomical elements, as well as the similarity in the reactions of the several chemical substances present, make it an exceedingly difficult matter to insure a complete isolation of the collagen. In a general way this may be readily done, but to obtain collagen from tendons, for example, absolutely free from all traces of mucin, elastin, and albuminous matter is not so easy. To be sure, the large proportion of collagen present in tendons practically insures, with reasonable care, freedom from any very large percentage of impurity ; but it is generally understood among physiological chemists that the sulphur of collagen or gelatin, for example, is somewhat questionable, that at least a portion of the 0.6 per cent of this element usually accredited to gelatin may be due to admixture of the richer sulphur-containing bodies, mucin or albumin, with which it is so intimately associated in the tissue. Indeed, it seems doubtful if we have on record any analysis of collagen or gelatin extracted from white fibrous connective tissue which can be accepted as representing the chemical composition of the perfectly pure substance. Thus, a glance through the literature of the subject shows that most of our knowledge, especially bearing on the chemical composition of gelatin, is based upon a study of the commercial product, the source of which is obviously more or less uncertain. It has therefore seemed to the writer very desirable, in view of the probable existence of a number of distinct collagens and gelatins, to determine as accurately as possible the chemical composition of gelatin from white fibrous tissue, having especially in view the preparation of the substance entirely free from all traces of elastin, mucin, and albumin. This seems the more important as a necessary step preliminary to comparing the reactions of

* Recherches sur la constitution des matières collagènes. Comptes rendus, t. lxxxii, p. 262.

gelatin with albumin,—a comparison which is of primary importance in determining the exact genetic relationship of these two bodies.

In many investigations on the chemical nature and properties of gelatin, the commercial product, purified perhaps by precipitation with alcohol, etc., has been employed and with valuable results, but in the light of present knowledge it seems quite imperative to establish with definiteness the chemical composition and reactions of gelatin from white fibrous connective tissue, and for this purpose it is necessary to have a product from a definite source and prepared in such a manner as to insure freedom from admixture, however slight, with any extraneous matters. The numerous proteid reactions obtained with ordinary gelatin are, in many cases at least, much less intense than those obtained with true proteids, a fact which has frequently led to the suggestion that gelatin owed its reactions in these cases to admixed albumin obtained from the tissue cells, etc.

When gelatin has once been prepared from the tissue-collagen, its further purification becomes exceedingly difficult. It is therefore of primary importance that the albumin, elastin and mucin be completely removed from the collagenous tissue prior to the conversion of the latter into gelatin. The removal of these substances, however, from so compact a tissue as tendon, with its predominance of collagenous fibers, is not easy, and considerable time must be given in order to accomplish this with any degree of thoroughness. The agents employed for the removal of these substances were water, half-saturated lime-water, dilute acetic acid, and alkaline pancreatic juice.

PREPARATION A.

Achilles tendons from oxen were carefully cleaned, freed from fat, etc., and then cut into very small pieces. The finely divided material (700 grams) was then soaked in thymolized water, frequently changed, for several days, to remove as much soluble matter as possible. It was next placed in 2 liters of

0.25 per cent solution of sodium carbonate; 300 c. c. of a powerful pancreatic juice, prepared from Kühne's dried pancreas, added, and the mixture warmed at 40° C. for five days, thymol being added from time to time to prevent putrefaction. In an alkaline pancreatic juice of this description the pieces of tendon swell up and the individual fibers become more or less separated, so that the mass becomes soft and spongy, thus giving the solvent a good opportunity to reach every part of the tissue.

As has been frequently demonstrated,* collagenous fibres which have not been boiled with water or treated with dilute acid are insoluble in an alkaline solution of trypsin. Elastic fibres, on the other hand, undergo digestion by this treatment, with formation of soluble products, elastoses and elastin-peptone. Mucin is likewise dissolved by the alkaline fluid, while all albuminous matter is readily converted into soluble proteoses, peptone, and amido-acids. This method would certainly seem to furnish an effectual means of removing all tissue-elements except the collagen; even the nucleo-proteids and nucleins of the tissue cells being dissolved by this treatment.

When the digestion was completed the residual collagen was strained off and washed thoroughly in large volumes of water until the pieces of tissue were without action on litmus paper and the fluid gave no reaction for dissolved proteids. It is to be noted, however, that when such apparently neutral tissue is boiled with water, the fluid gradually acquires an alkaline reaction, thus indicating that the last traces of alkali cannot be removed by water alone. The collagen was next boiled with several liters of water, each portion of water added being kept in contact with the collagen for a few hours only, in order to avoid the formation of any gelatoses by the too prolonged heating of the newly formed gelatin. For the same reason water was frequently added to prevent the boiling point of the solution being raised by the increasing concentration. When the first extract was poured off, the residual

* See Ewald, Zur Histologie und Chemie der elastischen Fasern und des Bindegewebes. Zeitschr. f. Biol., Bd. xxvi, p. 1.

collagen was boiled with a fresh quantity of distilled water, this operation being repeated until the larger part of the collagen was transformed into gelatin. The united fluids were then evaporated on the water-bath until quite concentrated, forming when cold a hard, firm jelly. The gelatin was next precipitated by alcohol, this being accomplished by pouring the warm jelly, with stirring, into a large volume of 95 per cent alcohol, by which it was thrown down as a soft stringy mass, gradually hardening by longer contact with the alcohol. The precipitate was then extracted for some days with absolute alcohol, and then with ether until it became hard and brittle. It was next ground up in a mortar, and the powder extracted continuously for two days with ether, for the complete removal of all fat or other ether-soluble matter. After this treatment the gelatin was dissolved in hot water, the fluid filtered through paper with the aid of a hot-water funnel, the filtrate concentrated somewhat, and again precipitated by a large volume of alcohol. The precipitate was dehydrated by repeated treatment with absolute alcohol, again pulverized and re-extracted with ether, after which it was considered ready for analysis.

For analysis, a sufficient amount of the substance was dried at 110° C. until of constant weight. Carbon and hydrogen were determined by combustion in an open tube with oxygen gas, the products of combustion passing over a layer of cupric oxide, a layer of lead chromate, and a roll of reduced copper. Nitrogen was determined by the Kjeldahl method, sulphur by fusion with sodium hydroxide and potassium nitrate in a silver crucible and precipitation as barium sulphate after the method recommended by Hammarsten.[*]

Following are the results obtained:

PREPARATION A.

I. 0.5359 gram substance gave 0.9818 gram $CO_2 = 49.96$ per cent C, and 0.3203 gram $H_2O = 6.64$ per cent H.

II. 0.3622 gram substance gave 0.6615 gram $CO_2 = 49.87$ per cent C, and 0.2145 gram $H_2O = 6.57$ per cent H.

[*] Zeitschr. f. physiol. Chemie, Bd. ix, p. 289.

III. 0.2789 gram substance gave 0.5118 gram $CO_2 = 50.04$ per cent C, and 0.1650 gram $H_2O = 6.57$ per cent H.

IV. 0.2006 gram substance gave 0.03565 gram $N = 17.77$ per cent N.

V. 0.1934 " " " 0.03435 " $N = 17.76$ " N.

VI. 0.9458 " " " 0.0214 " $BaSO_4 = 0.309$ per cent S.

VII. 0.8236 " " " 0.0202 " $BaSO_4 = 0.336$ " S.

VIII. 0.6468 " " " 0.0021 " ash $= 0.324$ per cent ash.

COMPOSITION OF THE ASH-FREE SUBSTANCE.

	I.	II.	III.	IV.	V.	VI.	VII.	Average.
Carbon,	50.12	50.03	50.21	...	...	...	...	50.12
Hydrogen,	6.66	6.59	6.59	...	...	...	...	6.61
Nitrogen,	...	...	...	17.82	17.86	...	...	17.84
Sulphur,	...	...	...	...	...	0.310	0.336	0.322
Oxygen,	...	...	...	...	...	...	...	25.10

PREPARATION B.

This preparation was made from calves' tendons, Achilles tendons, the method pursued being exactly similar to that made use of in preparation A.

The composition of the gelatin, dried at 110° C. until of constant weight, was as follows:

I. 0.2773 gram substance gave 0.5088 gram $CO_2 = 50.03$ per cent C, and 0.1597 gram $H_2O = 6.39$ per cent H.

II. 0.2711 gram substance gave 0.4957 gram $CO_2 = 49.86$ per cent C, and 0.1614 gram $H_2O = 6.61$ per cent H.

III. 0.6762 gram substance gave 1.2323 gram $CO_2 = 49.69$ per cent C, and 0.3934 gram $H_2O = 6.46$ per cent H.

IV. 0.4643 gram substance gave 0.8476 gram $CO_2 = 49.73$ per cent C, and 0.2734 gram $H_2O = 6.54$ per cent H.

V. 0.2469 gram substance gave 0.04409 gram $N = 17.85$ per cent N.

VI. 0.2105 " " " 0.03754 " $N = 17.79$ " N.

VII. 0.6945 " " " 0.0115 " $BaSO_4 = 0.226$ per cent S.

VIII. 0.7304 " " " 0.0127 " $BaSO_4 = 0.238$ " S.

IX. 1.0424 " " " 0.0180 " $BaSO_4 = 0.236$ " S.

X. 0.3090 " " " 0.0010 " ash $= 0.323$ per cent ash.

XI. 0.4839 " " " 0.0020 " ash $= 0.413$ " ash.

COMPOSITION OF THE ASH-FREE SUBSTANCE.

(Average ash $= 0.368$ per cent.)

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	Average.
Carbon,	50.21	50.04	49.87	49.91	...	...	...	...	...	50.01
Hydrogen,	6.41	6.63	6.48	6.56	...	...	...	...	...	6.52
Nitrogen,	...	...	...	...	17.91	17.85	...	...	...	17.88
Sulphur,	...	...	...	...	...	...	0.226	0.238	0.236	0.233
Oxygen,	...	...	...	...	...	...	...	...	...	25.35

PREPARATION C.

Here the method of preparing collagen was somewhat different from that followed in the two preceding cases. Ox tendons, carefully cleaned and cut into thin transverse sections with a razor (800 grams), were extracted thoroughly with water, then soaked in half-saturated lime-water for three days, for the removal of the mucin. The residual tissue was washed with water and again extracted with lime-water as before. After this treatment the tissue was washed repeatedly for five days with water slightly acidified with acetic acid, care being taken to avoid adding enough acid to cause swelling of the fibrous tissue. It was then digested with alkaline pancreatic juice at 40° C., 2.3 liters of an active solution of trypsin containing 0.25 per cent of sodium carbonate being employed. After five days' treatment of this kind the residual collagen was strained off, washed repeatedly with cold water until entirely free from alkaline reaction, after which it was converted into gelatin by the method already described, and further purified as detailed under Preparation A.

Analysis of the product, dried at 110° C. until of constant weight, gave the following results:

I. 0.3504 gram substance gave 0.6434 gram $CO_2 = 50.07$ per cent C, and 0.2086 gram $H_2O = 6.61$ per cent H.

II. 0.5127 gram substance gave 0.9390 gram $CO_2 = 49.94$ per cent C.

III. 0.3711 " " " 0.06601 " N = 17.78 " N.

IV. 0.1825 " " " 0.03245 " N = 17.78 " N.

V. 1.1582 " " " 0.0206 " $BaSO_4 = 0.243$ per cent S.

VI. 1.3220 " " " 0.0172 " $BaSO_4 = 0.177$ " S.

VII. 0.6631 " " " 0.0107 " $BaSO_4 = 0.221$ " S.

VIII. 0.5881 " " " 0.0017 " ash = 0.289 per cent ash.

IX. 0.2548 " " " 0.0009 " ash = 0.353 " ash.

COMPOSITION OF THE ASH-FREE SUBSTANCE.

(Average ash = 0.321 per cent.)

	I.	II.	III.	IV.	V.	VI.	VII.	Average.
Carbon,	50.23	50.10	. . .	. . .	. . .	. . .	. . .	50.16
Hydrogen,	6.63	. . .	. . .	. . .	. . .	. . .	. . .	6.63
Nitrogen,	. . .	. . .	17.83	17.83	. . .	. . .	. . .	17.83
Sulphur,	. . .	. . .	. . .	. . .	0.243	0.177	0.221	0.213
Oxygen,	. . .	. . .	. . .	. . .	. . .	. . .	. . .	25.14

PREPARATION D.

In this preparation the method of procedure was not quite so elaborate as in the three preceding cases. The tendons from oxen were cleaned and cut into thin transverse sections, soaked for some time in water, then extracted for five days with half-saturated lime-water, occasionally changed. The residual tissue was next washed for ten days with water slightly acidified with acetic acid, lastly with water alone. It was then boiled with water for about half an hour, the resultant solution being rejected, after which the collagen was transformed into gelatin as in the preceding cases, except that the boiling was not continued for so long a period, in order to avoid any hydration of the elastin that might be present. The gelatin was then purified as in the preceding cases.

Analysis of the dried product gave the following results:

I. 0.3019 gram substance gave 0.5528 gram $CO_2 = 49.93$ per cent C, and 0.1754 gram $H_2O = 6.45$ per cent H.

II. 0.3599 gram substance gave 0.6613 gram $CO_2 = 50.10$ per cent C, and 0.2107 gram $H_2O = 6.53$ per cent H.

III. 0.2181 gram substance gave 0.03865 gram N = 17.72 per cent N.

IV. 0.1649 " " " 0.02902 " N = 17.60 " N.

V. 0.6478 " " " 0.0111 " $BaSO_4 = 0.234$ per cent S.

VI. 1.2868 " " " 0.0241 " $BaSO_4 = 0.256$ " S.

VII. 1.2868 " " " 0.0270 " $BaSO_4 = 0.287$ " S.

VIII. 0.3640 " " " 0.0010 " ash = 0.274 per cent ash.

IX. 0.4274 " " " 0.0013 " ash = 0.304 " ash.

Composition of the Ash-free Substance.

(Average ash = 0.289 per cent.)

	I.	II.	III.	IV.	V.	VI.	VII.	Average.
Carbon,	50.07	50.24	. . .	. . .	. . .	. . .	. . .	50.15
Hydrogen,	6.46	6.54	. . .	. . .	. . .	. . .	. . .	6.50
Nitrogen,	. . .	. . .	17.77	17.65	. . .	. . .	. . .	17.71
Sulphur,	. . .	. . .	. . .	. . .	0.234	0.256	0.287	0.259
Oxygen,	. . .	. . .	. . .	. . .	. . .	. . .	. . .	25.38

Average Composition of the Different Preparations, Ash-free.

	A.	B.	C.	D.	Average.
Carbon,	50.12	50.01	50.16	50.15	50.11
Hydrogen,	6.61	6.52	6.63	6.50	6.56
Nitrogen,	17.84	17.88	17.83	17.71	17.81
Sulphur,	0.322	0.233	0.213	0.259	0.256
Oxygen,	25.10	25.35	25.14	25.38	25.24
Ash,	0.324	0.368	0.321	0.289	0.325

The results of these analyses agree so closely that it is safe to conclude that the several preparations have practically the same composition. Especially noticeable is the low content of sulphur and the small content of ash, two features which may be taken as indicative of the great purity of the products. Undoubtedly the small percentage of sulphur, as contrasted with those in the older analyses, is due to the more complete removal of mucin in these preparations, for, as has been recently found, the sulphur-content of mucin from tendons is quite high, 2.32 per cent.* It is also to be noted that the present analyses show the percentage of sulphur in this form of gelatin to be even lower than the amount found by Mörner in the gelatin obtained from corneal cartilage, 0.3 per cent. Further, the above samples of gelatin give no reaction for mercaptan-sulphur with plumbic acetate and potassium hydroxide.

In preparation C, phosphorus was determined by fusion with sodium hydroxide and potassium nitrate, etc. The result showed the presence of 0.025 per cent of phosphorus. The ash, however, contained calcium phosphate. Calculating all the phosphorus found to tribasic calcium phosphate would account for less than half the ash. The ash also contained calcium sulphate. Taking the average content of ash and calculating half of it as calcium sulphate would require 0.038 per cent of sulphur, an amount too small to materially modify the percentage of sulphur found in the substance.

Among the earlier analyses of gelatin, or rather of collagen, from white fibrous connective tissue are those of Scherer.† His process of preparation consisted in macerating the tendons in water containing saltpetre, washing repeatedly with water, and finally extracting with alcohol and ether. The average composition of the product was C 50.51, H 7.16, N 18.37, S and O 24.03.

Hofmeister ‡ prepared a body which he considered identical

---

* Chittenden and Gies, The Mucin of White Fibrous Connective Tissue. Journal of Exper. Medicine, vol. i, p. 186.

† Annal. d. Chem. u. Pharm., Bd. xl, p. 46.

‡ Ueber die chemische Structur des Collagens. Zeitschr. f. physiol. Chem., Band ii, p. 299.

with natural collagen, by heating commercial gelatin (purified by precipitation with alcohol and containing 0.61 per cent of ash) to 130° C. until constant weight was attained. This substance he believed to differ from the gelatin by only one molecule of water. Its composition was C 50.75, H 6.47, N 17.86, S and O 24.92. It contained 0.62 per cent of ash. Sulphur was not determined.

Gorup-Besanez * gives the following analysis of tendons and of the gelatin prepared from them:

	Tendons.	Gelatin.
Carbon,	50.9	50.2
Hydrogen,	7.2	6.7
Nitrogen,	18.3	17.9
Sulphur, ⎱ Oxygen, ⎰	23.5	25.0

The content of sulphur in gelatin he gave as 0.56 per cent.

Chittenden and Solley,† by an analysis of commercial gelatin, purified by precipitation with alcohol, etc., obtained the following results: C 49.38, H 6.81, N 17.97, S 0.71, O 25.13. The ash content was 1.26 per cent.

Hammarsten ‡ obtained 0.665–0.747 per cent of sulphur in commercial gelatin containing 1.74 per cent of ash.

Weiske § prepared gelatin or gelatoses from bone by extracting with dilute hydrochloric acid and then heating the residual tissue with water at 130° C. in a Papin's digester. This product contained only 0.30 per cent of ash. The disadvantage of extracting tissue of this kind with hydrochloric acid, although unavoidable in the study of bone-collagen, is apparent from his own statement that after washing the softened tissue in water for four weeks it still contained chlorine. From commercial gelatin, according to O. Nasse,‖ the inorganic salts may be removed by long-continued soaking in water. In this way he was able to reduce the ash to 0.6

---

* Lehrbuch d. physiol. Chem., 1878, p. 142.

† The Primary Cleavage Products formed in the Digestion of Gelatin. Journal of Physiol., vol. xii, p. 23.

‡ Zeitschr. f. physiol. Chem., Band ix, p. 305.

§ Zur Chemie des Glutins. Ibid., Bd. vii, p. 460.

‖ Ueber die Chemie des Glutins. Jahresbericht d. Thierchemie, 1889, p. 29.

per cent. The soaking of gelatin in dilute acid, however, results in the formation of a salt-like compound, from which the acid cannot be withdrawn by washing with water. By adding a little ammonia to the water this may be accomplished.

From this short résumé it is evident that pure gelatin from white fibrous connective tissue is not widely different in composition from commercial gelatin, so far as the content of carbon, hydrogen, and nitrogen is concerned. In the content of sulphur, however, there is a wide difference, pure gelatin from tendons containing only 0.25 per cent of this element. Especially important in this connection is the fact, now brought out, that the sulphur-content of gelatin from white fibrous connective tissue is very closely akin to that of the related albuminoid elastin,* the latter, according to the latest researches, containing 0.3 per cent of this element. Elastin, however, differs widely from gelatin in its content of carbon and nitrogen. Further, the fancied difference in the content of sulphur between corneal gelatin and tendon gelatin also falls away, although these bodies are plainly unlike in their content of nitrogen.

REACTIONS OF GELATIN.

Although the four samples of gelatin described were studied with great care, no difference in chemical reactions was observed. They all showed a very faint alkaline reaction and were rather noticeably soluble in cold water. In trying precipitations with various reagents, a 2 per cent aqueous solution of gelatin was made use of, and at room temperature this was fluid enough for the purpose, but if warmed and allowed to cool undisturbed the solution showed some tendency to gelatinize. Halliburton † states that the power of gelatinizing is possessed by solutions from 1 per cent up, but diminishes with every successive solution. This somewhat weak power

---

* Chittenden and Hart, Elastin and Elastose Bodies. Studies in Physiol. Chem., Yale Univer., vol. iii, p. 19. Also Schwarz, Untersuchungen über die chemische Beschaffenheit der elastischen Substanz der Aorta. Zeitschr. f. physiol. Chem., Bd. xviii, p. 487.

† Text-book of Chem. Physiol. and Pathol., p. 471.

of gelatination is not to be ascribed to extensive conversion into gelatoses, but is more probably connected with the greater purity of the products. Thus O. Nasse * observed that the more nearly free from ash his gelatin was, the less the tendency to gelatinize, diminution in the percentage of ash leading to loss of the power of gelatination. This being the case, freedom from inorganic constituents might naturally be expected to result in greater solubility in cold water. Hammarsten † mentions the well-known fact that gelatin is more easily precipitated by alcohol in the presence of neutral salts, and he further mentions that " it is precipitated by tannic acid in the presence of salt." Salts unquestionably do exert a very marked influence in rendering precipitation of gelatin more complete, from which it might be argued that their absence from or diminution in gelatin would enhance its solubility in water and decrease its power of gelatinizing. On the other hand, it is of course possible that the long-continued process of preparation may have resulted in the formation of some gelatose, although great care was exercised to avoid such an occurrence, the heating with water in the conversion of collagen into gelatin having been of comparatively short duration.

In the precipitation of pure gelatin with most reagents there is noticeable a tendency toward incompleteness of separation and a dependence of the reaction upon the attendant conditions, especially the concentration of the fluid. Upon this fact, no doubt, many of the conflicting statements in regard to the reactions of gelatin depend, although some of the differences noted by various writers are due to the lack of purity of the gelatin employed, and possibly also to the source from which it was derived. Further, the fact that with certain reagents only slight precipitates are obtained has led to the belief that these reactions are due to the presence of albumin and not to gelatin itself, whereas the correct explanation in many cases is to be found in the solubility of the

* Jahresbericht d. Thierchemie, 1889, p. 31.
† Lehrbuch d. physiol. Chem., 1895, p. 47.

precipitate, in the excess of the reagent, for example, which may even prevent its appearance altogether if the conditions are not favorable.

Pure gelatin gives the biuret or Piotrowski's reaction strongly, the shade of color being a violet-purple. Millon's and the xanthoproteic reactions are obtained with great distinctness, the former being especially strong, although in both cases the reaction is by no means as marked as that obtained with corresponding amounts of albumin or globulin. The reactions are altogether too pronounced to be credited to traces of contained albumin, as has frequently been done in the past. Far more plausible is it to assume that the radical or radicals (perhaps an oxybenzene group) to which these reactions are due, and which are so abundant in the proteid molecule, are contained only in small proportion in the gelatin molecule.

With Adamkiewicz's reaction, and also with Liebermann's reaction, pure gelatin yields negative results.

With acetic acid and potassium ferrocyanide a slight precipitation is obtained, if the ferrocyanide solution is added carefully. A very slight excess of the latter redissolves the precipitate.

With picric acid, trichloracetic acid, tannic acid, chloroplatinic acid, phosphomolybdic acid and phosphotungstic acid, pure gelatin is more or less completely precipitated. With picric acid the precipitate first formed redissolves readily, becoming permanent on addition of more of the reagent. It is somewhat soluble in excess of the precipitant. With trichloracetic acid the precipitation is far from complete.

The following reagents do not precipitate pure gelatin: plumbic acetate, basic lead acetate, cupric sulphate, ferric chloride, silver nitrate, and mercuric chloride.

Mercuric chloride, however, causes a precipitate if hydrochloric acid is added. As solutions of mercuric chloride sometimes contain a little free acid, a turbidity may be obtained with the mercuric chloride alone. The precipitate formed on adding acid is very soluble in excess of acid, especially on

warming the fluid, reappearing as the solution cools. Sodium chloride appears to assist in the precipitation if added in considerable quantity (in 20 per cent solution), simply by making a medium in which the precipitate is less soluble, but it does not play any essential part in the reaction. Acetic acid will not take the place of hydrochloric acid in this reaction, neither will sodium chloride induce precipitation of gelatin by mercuric chloride in the absence of acid.

Gelatin is precipitated from aqueous solutions by saturation of the fluid with ammonium sulphate, with sodium sulphate, and with magnesium sulphate.

Saturation with sodium chloride causes only a slight precipitate, but if acetic acid is added to the salt-saturated fluid a heavy flocculent precipitate results. Hydrochloric acid will take the place of acetic acid in this reaction, but the precipitate is more soluble in excess of the acid.

Saturation with ammonium chloride fails to produce any precipitate until the fluid is acidified with acetic acid, when the gelatin, in part at least, is thrown down.

A FURTHER STUDY OF THE INFLUENCE OF ALCO-HOL AND ALCOHOLIC DRINKS UPON DIGESTION, WITH SPECIAL REFERENCE TO SECRETION.*

By R. H. CHITTENDEN, LAFAYETTE B. MENDEL AND HOLMES C. JACKSON.†

IN a previous paper ‡ on the " Influence of Alcohol and Alcoholic Drinks upon the Chemical Processes of Digestion " it was carefully pointed out that any complete and satisfactory answer to the question " How do alcoholic fluids affect digestion? " cannot be obtained by any single line of experimentation, since the rate and extent of digestion may be modified in a variety of ways and through a variety of channels. Thus, due consideration must be given not only to the direct influence of alcoholic fluids upon the solvent or digestive power of the several digestive juices, but heed must also be given to the quantitative and qualitative modifications which the secretions themselves may undergo, as well as to variations in the rate of absorption and to the possible inter-action of these and other factors. In our earlier paper, the data presented threw light only upon the character and extent of the influence exerted by various alcoholic fluids upon the purely chemical processes of digestion, *i. e.*, upon amylolysis and proteolysis. In the continuation of these studies during the past year our efforts have been directed mainly to acquiring a fuller knowledge of the action of alcoholic beverages upon secretion; and in so doing new data

---

* Being a statement of some research work done for the Committee of Fifty for the Investigation of the Liquor Problem, and to be regarded as a preliminary report, contributing facts upon which a general discussion may in the future be undertaken by the Committee as a whole.

† Reprinted from the American Journal of Physiology, vol. i.

‡ Chittenden and Mendel, American Journal of the Medical Sciences. 1896. January–April.

have been obtained which, it is hoped, will prove of value in explaining more fully the action of these fluids upon the whole process of digestion.

SALIVARY SECRETION.

The current statements regarding the influence of alcohol on the secretion of saliva are confined to a brief reference to the direct action on the flow into the mouth. Thus it is stated that almost coincident with the burning sensation caused by alcohol taken into the mouth, a copious flow of saliva begins, due to reflex stimulation of the glands through the nervous system.[*] We have performed experiments with the object of ascertaining (1) the possible variations in the amount of salivary flow due to the presence of alcoholic fluids in the mouth, psychical influences being eliminated so far as possible; (2) the character of the saliva thus secreted; (3) the influence upon secretion of alcoholic beverages introduced into the stomach. It seemed particularly desirable to investigate this latter phase in view of the asserted influence of irritating substances (vinegar, alcoholic extract of pepper, etc.) when introduced directly into the alimentary tract through a fistula. There is said to result under such conditions a reflex flow of saliva, the nervous impulses being transmitted through the vagus.[†]

The Influence of Alcoholic Fluids introduced into the Mouth. — In the following experiments the attempt was made to ascertain something as to the character and extent of the direct stimulation of the salivary glands provoked by the presence of alcoholic fluids in the mouth, as well as to determine what quantitative changes, if any, may be called forth in the composition of the secretion in this way. These experiments were made on both man and dogs. The method, in the first instance, consisted in taking into the mouth

[*] Compare, for example, Kühne, Lehrbuch der physiol. Chemie, 1868, p. 2; Lauder Brunton, Disorders of digestion, 1886, p. 143.

[†] Oehl, Comptes rendus, lix, p. 336, quoted by Heidenhain, Hermann's Handbuch der Physiologie, 1883, v, p. 83.

10 c. c. of the fluid studied, and allowing it to remain there for an instant previous to swallowing it. The normal conditions were thus closely imitated, and reflex influences from the stomach not excluded. The head was now turned to one side and rested upon the arm, the saliva being allowed to collect in the cavity of the mouth. As the fluid accumulated it was from time to time, during fifteen to twenty minutes, allowed to flow out of a corner of the mouth into a measuring vessel. Movements of the jaws and tongue were carefully avoided and psychic stimulation was excluded as far as possible. The method, already recommended by Hofbauer,* was found to be reasonably satisfactory, and control trials showed that the quantities of saliva obtained within periods of fifteen or twenty minutes could be appropriately compared.

Of the saliva thus collected, 3–4 c.c. were taken for analysis. A weighed quantity was dried in a tared crucible on a water-bath and then for four or five hours at 105°C., this time being found sufficient to bring crucible and contents to a constant weight. Total solids were thus determined. The crucible was then ignited, care being taken to prevent loss by volatilization of salts. The ash thus obtained is given as salts in the protocols, while the organic constituents were obtained by subtracting the amount of salts from the total solids. In some cases the amount of chlorine in the ash was determined by the usual method of titration with weak silver nitrate solution. The analytical results are all expressed in percentages. The following figures serve to illustrate the results of a typical duplicate analysis : —

SUBMAXILLARY SALIVA OF DOG.

	Water.	Total Solids.	Organic Constituents.	Salts.	Chlorine.
A.	98.99	1.01	0.80	0.21	0.042
B.	98.99	1.01	0.78	0.23	0.040

* Hofbauer, Archiv für die ges. Physiol., 1897, lxv, p. 503.

It is an observation easily verified, that the presence of a small quantity of strong alcohol or alcoholic beverage in the mouth excites a sudden flow of saliva. This acceleration in flow is, at most, a very brief one, and the rate of flow quickly returns to that pertaining to normal conditions, *i. e.*, absence of stimuli in the mouth. The stimulation in this case is not due merely to the mechanical action of the fluid introduced, nor is it a form of stimulation specific for alcohol alone, as our experiments on dogs have demonstrated. Thus, animals were anæsthetized with ether and chloroform through a tracheal cannula (thereby avoiding direct stimulation of salivary flow), a small dose of morphine, or a larger one of chloral, having been previously administered. A cannula was then introduced into one or both ducts of the submaxillary glands. A small wad of absorbent cotton moistened with the fluid to be studied was introduced with a forceps into the back of the mouth upon the tongue, and the flow of saliva from the ends of the cannulas noted. It was found by this method that water or weak sodium chloride solution (0.7 per cent) produced no further effect than the secretion of a drop or two of saliva due to the mere mechanical action of introducing the wad; with increasing strengths of salt the secretion was decidedly accelerated, flowing readily after application of 20 per cent salt solution, the acceleration, however, being very brief in duration (5 min.). The buccal cavity could be swabbed out with water occasionally, the effect being a minimal one. It was found that *weak* alcohol, introduced in this way, provoked little, if any, flow; while stronger alcohol (50 per cent) gave rise to a transitory secretion, the stimulation in this case, however, being far more marked than can be produced by the indirect action of alcohol through the stomach. Thus, in one animal, in which the activity of the glands was found pronounced when a drop of dilute acetic acid was applied to the tongue, injection of 100 c.c. 50 per cent alcohol directly into the stomach, failed to provoke any reflex salivary flow in half an hour.

Turning now to the influence of alcoholic fluids upon the

SALIVARY EXPERIMENTS ON MAN.

	I.		II.		III.		IV.		V.		VI.		VII.		VIII.	
	Water. a	Water. b	Water. a	Water. b	Water. a	Brandy. b	Water. a	Brandy. b	Water. a	Brandy. b	Water. a	Brandy. b	Water. a	Gin. b	Water. a	Sherry. b
Amount collected in c.c. per 10 minutes.	4.00	4.00	4.40	3.70	2.70	5.30	3.80	4.40	4.70	8.00	4.40	7.10	4.00	4.60	3.50	4.40
Water, per cent.	99.49	99.57	99.52	99.54	99.51	99.49	99.50	99.40	99.57	99.19	99.56	99.45	99.57	99.51	99.41	99.39
Total solids, per cent.	0.51	0.43	0.48	0.46	0.49	0.51	0.50	0.60	0.43	0.81	0.44	0.55	0.43	0.49	0.59	0.61
Organic constituents, per cent.	0.38	0.31	0.35	0.33	0.33	0.35	0.35	0.45	0.31	0.58	0.30	0.38	0.31	0.35	0.41	0.43
Salts, per cent.	0.15	0.12	0.13	0.13	0.16	0.16	0.15	0.15	0.12	0.23	0.14	0.17	0.12	0.14	0.18	0.18
Salts calculated on total solids, per cent.	29.00	29.00	28.00	28.00	32.00	32.00	30.00	25.00	29.00	28.00	31.00	31.00	28.00	28.00	30.00	29.00

rate of flow and composition of the saliva in man, the accompanying experiments, by the method above indicated, may be cited (p. 77). The first two (I and II) show the results obtained with successive portions of water; in the following ones, a control experiment with water in each instance preceded the trial with the alcoholic fluid.

The alcoholic content of the fluids employed was as follows: Brandy, 47 per cent by vol.; gin, 51 per cent; sherry, 21 per cent.

From these figures it is seen that the results obtained with two successive portions of water scarcely differ from each other, the tendency, however, being towards decreased flow accompanied by decrease in dissolved material in the saliva. Interpreted in physiological terms, these results indicate that the second stimulation with water is, if anything, weaker than its predecessor. In decided contrast appear the results obtained with the alcoholic liquors. Here may be observed an increased flow of saliva, not pronounced, but accompanied by an increase in both organic and inorganic constituents. The effect is precisely analogous, both in composition and rate of flow, to that brought about by an increase in intensity of stimulation, when the salivary glands are electrically excited through their nerves.[*]

The following diagram represents in graphic form the results given in the preceding table, i. e. (1), the relative rate of flow induced by water and by the alcoholic fluid; (2) the content of solid matter, together with the relative proportion of ash or inorganic matter and of organic matter as indicated by the loss on ignition.

The Influence of Alcoholic Liquors introduced directly into the Stomach. — In our experiments on the reflex stimulation of salivary flow, the attempt to produce a persisting secretion due to the presence of alcohol in the stomach was unsuccessful; nor have we been able to obtain evidence of an unusual flow

---

[*] Cf. Heidenhain: Archiv für die ges. Physiol., 1878, xvii, p. 7, and Hermann's Handbuch der Physiologie, v, p. 52.
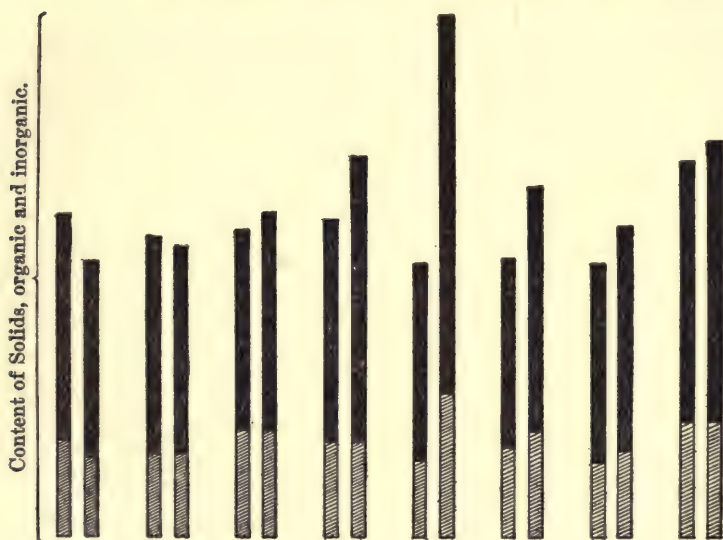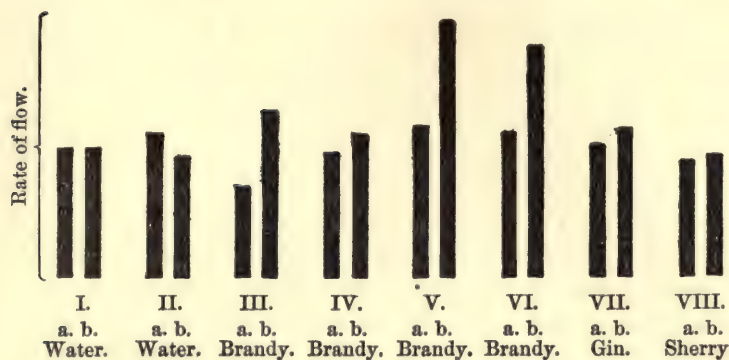
Diagram illustrating the relative influence of alcoholic fluids on the rate of secretion and composition of human saliva.

of saliva under such circumstances in dogs with gastric fistulæ. It seemed desirable, however, to examine the possible direct influence of alcoholic fluids on the salivary glands and the resulting secretion, when other factors were excluded as far as possible. In these experiments dogs of 10 to 18 kilos were used. Chloroform-ether mixture was employed to produce

anæsthesia, and was administered through a tracheal tube in part of the experiments, the danger of respiratory difficulties resulting from salivary flow induced in the glands as a result of the ether stimulation being thus avoided. In the later stages of the experiments the alcohol introduced usually sufficed to maintain the animal in perfect quiet. Fredericq * has recommended the use of alcohol for producing narcosis in rabbits; it has been found quite satisfactory for this purpose in the dog, the effects passing off with relative rapidity.

A glass cannula, bent at the end, was tied in Wharton's duct (and occasionally a second cannula into the duct of the sublingual gland). The chordo-lingual nerve was ligatured and cut at some distance centrally to the point where the chorda tympani branches off to the glands. All secretion in the corresponding gland was thus stopped except during stimulation of the chorda, which was accomplished through raising the peripheral end of the cut nerve by the ligature and slipping hook electrodes under it. The interrupted current of a Du Bois induction-coil with a single element was used as the stimulus. Saliva was collected in small graduated cylinders. Alcohol was introduced into the stomach by making an incision through the linea alba, etc., and injecting the fluid directly into the organ thus exposed by means of a large needle-pointed syringe. By careful avoidance of the larger gastric vessels, bleeding was minimal. The general course of the experiments was as follows : A distance between the primary and secondary coil of the inductorium was selected such as a preliminary trial showed to give a medium rate of flow. This stimulus was, so far as possible, kept constant throughout the experiment. The chorda was repeatedly stimulated for periods of one minute, followed by pauses of two minutes, during which the nerve was kept covered. In this way sufficient quantities of saliva for analysis were collected. Before collecting a sample of saliva under any given conditions, the six or seven drops first discharged were thrown away, and thus the fluid stored up from previous stimulation in the

* Fredericq : Manipulations de physiologie, p. 19.

gland lumina, ducts, and cannula was avoided.* After collecting two or three control samples, the fluid to be considered (usually warmed slightly) was injected into the stomach in the manner already described, and this was followed by a pause of five minutes. The pulse was observed at frequent intervals to detect any possible influence on the heart's action and consequent blood-flow. The samples of saliva collected were analyzed in the manner already described. At the conclusion of the experiment, the animal was killed by bleeding, and the condition of the glands, as well as of the stomach and other organs, examined. The protocols of three typical experiments are given below.

1, iii, 1897. Dog. Weight 14 kilos. Chloroform and ether administered through tracheal tube during part of the experiment. Distance of secondary coil = 200 mm. Period of stimulation = 1 min., followed by a pause of 2 min.

	Time.	Amount Saliva Collected.	Rate of Secretion per min.	Water.	Total Solids.	Organic Matter.	Salts.	Chlorine.
		c.c.	c.c.	per cent.	per cent.	per cent.	per cent.	per cent.
I†	10.35	5.3	0.7	98.76	1.24	0.94	0.30	0.026
II	11.08	5.4	0.6	98.94	1.06	0.73	0.33	0.036
III	11.38	5.2	0.8	98.95	1.05	0.69	0.36	0.044
IV	11.56	4.0	0.8	98.90	1.10	. . .	. . .	0.048
	12.20	80 c.c. 50 per cent alcohol injected into stomach.						
V	12.35	4.8	0.8	98.96	1.04	0.69	0.35	0.047
VI	12.55	4.8	0.8	99.01	0.99	0.59	0.40	0.076
	1.15	100 c.c. 50 per cent alcohol injected into stomach.						
VII	1.21	4.9	0.8	99.05	0.95	0.59	0.36	0.055
VIII	1.42	6.0	1.0	99.05	0.95	0.60	0.35	0.060
IX	2.02	5.5	0.9	99.14	0.86	0.52	0.34	0.048
X	2.24	5.2	0.8	99.17	0.83	0.47	0.36	0.042
	2.53	100 c.c. 50 per cent alcohol injected into stomach.						
XI	2.58	4.5	0.6	99.07	0.93	0.63	0.30	0.034
XII	3.27	6.0	0.6	99.18	0.82	0.53	0.29	0.037
XIII	4.10	5.0	0.7	99.17	0.83	0.49	0.34	0.038

Dog killed. Stomach mucosa normal in appearance. Urinary bladder and gall bladder greatly distended. Stomach contents = 450 c.c., faintly acid in reaction, and containing 24.6 grams of alcohol. No food present.

---

* Cf. Heidenhain, Hermann's Handbuch der Physiologie, v, p. 53; Langley and Fletcher; Philosophical Transactions, 1889, clxxx., B., p. 112.

† In this first period the distance of the secondary coil was 280 mm., but the stimulation was unsatisfactory.

22, iii, 1897. Bitch. Weight, 10 kilos. Chloroform and ether administered during operation. Tracheotomy performed after operation. Distance of secondary coil = 240 mm. Period of stimulation = 1 min., followed by 2 min. pause.

	Time.	Amount Saliva. Collected.	Rate of Secretion per min.	Water.	Total Solids.	Organic Matter.	Salts.	Chlorine.
		c.c.	c.c.	per cent.	per cent.	per cent.	per cent.	per cent.
I	11.30	4.6	1.1	98.68	1.32	1.03	0.29	0.032
II	11.42	4.7	0.9	98.70	1.30	0.96	0.34	0.074
III	11.57	4.0	0.7	98.84	1.16	0.73	0.43	0.146
	12.35	150 c.c. burgundy injected into stomach.						
IV	12.41	4.9	0.8	98.72	1.28	0.91	0.37	0.092
V	12.59	5.5	0.6	98.78	1.22	0.87	0.35	0.096
VI	1.29	4.7	0.7	98.91	1.09	0.82	0.27	0.071
	2.00	200 c.c. burgundy injected into stomach.						
VII	2.06	4.7	0.6	98.88	1.12	0.82	0.30	0.058
VIII	2.32	. . .	. . .	98.98	1.02	0.69	0.33	0.099

Dog killed; stomach contents = 190 c.c.; claret color; mucosa not inflamed. Contents contained 13.1 grams of alcohol. The burgundy used contained 5.2 per cent of alcohol.

12, iv, 1897. Bitch. Weight 9 kilos. Chloroform and ether during operation. Distance of secondary coil = 190 mm. Stimulation 1 min., followed by a pause of 2 min.

	Time.	Amount Saliva Collected	Rate of Secretion per min.	Water.	Total Solids.	Organic Matter.	Salts.	Chlorine.
		c.c.	c.c.	per cent.	per cent.	per cent.	per cent.	per cent.
I	9.24	4.5	0.9	98.76	1.24	0.97	0.27	0.062
II	9.40	4.6	0.7	98.89	1.11	0.81	0.30	0.054
	10.40	100 c.c. distilled water injected into stomach.						
III	10.53	4.7	0.6	99.04	0.96	0.66	0.30	0.049
IV	11.21	5.0	0.5	99.09	0.91	0.60	0.31	0.060
	11.50	100 c.c. distilled water injected into stomach.						
V	11.56	4.5	0.5	99.30	0.70	0.54	0.16	0.024
VI	12.25	4.5	0.6	99.33	0.67	0.36	0.31	0.078
VII	12.51	4.6	0.7	99.39	0.61	0.36	0.25	0.063
	1.18	100 c.c. 50 per cent alcohol injected into stomach.						
VIII	1.23	5.7	0.7	99.35	0.65	0.36	0.29	0.067
IX	1.44	4.8	0.8	99.38	0.62	0.32	0.30	0.087
X	2.03	4.7	0.7	99.47	0.53	0.29	0.24	0.087
XI	2.25	4.7	0.6	99.47	0.53	0.22	0.31	0.097

Dog killed. Stomach mucosa normal. Contents = 100 c.c. No odor of alcohol.

Experiments of the character indicated by these protocols were carried out with alcohol in varying doses, whisky, brandy, and wine, and control experiments with water were also made. In attempting to interpret the analytical data thus obtained in experiments extending over several hours it is necessary to bear in mind facts regarding salivary secretion which seem to be sufficiently established. Ludwig [*] showed that the submaxillary saliva secreted during stimulation of the chorda tympani undergoes a change in composition varying with the duration of the flow, the content of organic solids decreasing in far greater degree than the dissolved salts. Heidenhain [†] found that the percentage of salts in the saliva varies directly with the rate of secretion, quite independently of the state of the gland, the organic constituents, however, being influenced by the condition of the secreting organ as well as by the strength of stimulus and resulting rate of secretion. These observations, verified by Werther [‡] and by Langley and Fletcher, [§] have been extended by the latter investigators, who formulated the opinion that "the secretion of organic substances depends wholly, or almost wholly, upon the strength of the stimulus, whilst the secretion of water and of salts depends also upon the amount of blood flowing through the gland." [||] In view of the well-known fact that changes in the strength of the stimulus immediately bring about a change in both rate of secretion and composition of the saliva, we have attempted to maintain a constant stimulus throughout each series of observations by selecting some satisfactory distance of the secondary coil of the inductorium and by applying the electrodes as uniformly as possible. Owing to the gradual decline in the irritability of the exposed nerve, the impossibility of applying the electrodes constantly in one position, and other unavoidable difficulties, ideal results cannot be obtained. However, the difficulties were present in

---

[*] Ludwig and Becher, Zeitschr. f. rat. Med., 1851, N. F. i, p. 278. Cf. also Heidenhain, Hermann's Handbuch der Physiologie, v, pp. 47–49.

[†] Heidenhain, Archiv für die ges. Physiol., 1878, xvii, p. 4 and 6.

[‡] Werther, Archiv f. d. ges. Physiol., 1886, xxxviii, p. 293.

[§] Langley and Fletcher, loc. cit., p. 152.   [||] Ibid., p. 132.

every experiment and the results are therefore more or less comparable.

An examination of the data obtained in the manner above indicated shows no constant appreciable influence of alcohol or alcoholic fluids upon the *rate of secretion* of submaxillary (or sublingual) saliva under the influence of a constant external stimulus. Even large doses of alcohol, sufficient to produce prolonged narcosis, fail to check the salivary flow, a result in striking contrast to the effects which morphine may bring about when used in moderately large doses. We have not infrequently observed, in other experiments, an entire absence of salivary flow even with very strong stimuli, when morphine was unintentionally given in doses larger than were necessary to produce a mild narcosis. On the other hand, there is likewise an absence of any stimulating action on the glands, in our experiments; at least the slight variations in the rate of flow after alcohol is administered are no greater than those brought about by water alone (cf. third protocol above). On the *total solids* likewise, the presence of alcohol seems to exercise no noticeable influence. There is a tendency toward decrease in amount as the experiments progress; this decrease, however, is entirely confined to the *organic constituents* of the saliva, the *salts* remaining comparatively constant in amount, as can be seen in the protocols above. The decrease in organic substances is in no way to be attributed to alcohol, since it may be obtained with water alone (cf. protocol third), or in the course of any protracted salivary secretion. Nor is this decrease remarkable when it is remembered that a small gland weighing a few grams has furnished 50 to 75 grams of saliva in the course of three or four hours. The organic constituents of the cells must thus be exhausted somewhat more rapidly than the anabolic processes of the gland can replace them, while the salts are obtained with relative ease from the blood. Any effect upon the secretion of inorganic salts such as might result in accordance with Langley's law (cf. p. 83) was not observed. A large number

of determinations of the alkalinity of the saliva (towards lacmoid) likewise failed to show any constant relations. It is interesting in this connection to note that the submaxillary saliva of the dog was always found alkaline to phenol-phthalëin, litmus, lacmoid, and methylorange. Mixed human saliva, like the bile of a number of animals, is almost always acid toward phenolphthalëin.*

GASTRIC SECRETION.

It has already been pointed out that in an accurate and complete study of the influence of alcohol and alcoholic drinks upon gastric digestion, no single line of experimentation can lead to full and concise results covering the whole ground of inquiry. It was therefore deemed advisable, for experimental purposes, to study the subject under several distinct heads, as (1) the influence of alcohol and alcoholic drinks upon the process of secretion; (2) upon the processes of absorption; (3) upon the motor functions of the alimentary canal; and (4) upon the purely chemical processes of gastric digestion. The last phase has already been considered at some length.†

The older announcements regarding the influence of alcohol are summarized in the statement that it is a strong stimulant of gastric secretion, and alcohol is recommended as a means of obtaining gastric juice from fistulæ in animals.‡ Larger doses are regarded as detrimental to the stomach, giving rise to transudation of alkaline fluid, — a process evidently path-ological.§ Gluzinski ‖ found in experiments on man with brandy and dilute alcohol that these liquors gave rise, after

---

* Chittenden, The Reactions of some Animal Fluids. Science, N. S., v, p. 902.

† Chittenden and Mendel, loc. cit.

‡ Cf. Frerichs, Wagner's Handwörterbuch der Physiologie, 1846, iii (1), p. 788; Kühne, Lehrbuch, pp. 28, 30; Heidenhain, Hermann's Handbuch der Physiologie, v, p. 115.

§ Cf. Heidenhain, loc. cit.; Lauder Brunton, Disorders of digestion, 1886, p. 144.

‖ Gluzinski, Deutsches Archiv f. klin. Med., 1886, xxxix, p. 405. See Jahresbericht für Thierchemie, 1886, xvi, p. 263.

a brief preliminary period, to the formation of a very active secretion rich in hydrochloric acid.

Likewise Wolff * states that cognac in small dose increases the secretion of hydrochloric acid, while in larger quantity it decreases the acidity of the gastric juice and retards peptone formation. The stomach fails to respond in a positive way, however, after the continued use of alcohol. While Klemperer † failed to note more than a very slight increase in secretion resulting from moderate doses of alcohol, Blumenau ‡ observed that 25–50 per cent alcohol introduced into the healthy human stomach acts as a secretory stimulant, bringing about an increased flow of gastric juice with rise of acidity after a period of 2–3 hours. More recently Brandl § has found in experiments on fistulous dogs that alcohol — as contrasted with water introduced with food stuffs into the stomach — brings about an unfailing, though not particularly large, increase in gastric secretion. With repeated and increasing doses of alcohol, Haan ‖ has further observed an augmentation of acidity in the dog, followed by a diminution in the amount of secretion and a gradual decline in acidity after several doses.

In our first series of experiments on gastric secretion, attention was directed to the volume and acidity resulting from the introduction of alcoholic fluids into the stomach, independently of any stimulating action due to food simultaneously introduced. Dogs in fasting condition were employed in every instance, and morphine sulphate (introduced subcutaneously) followed by chloroform-ether was used preparatory to operative interference. The method consisted in ligating the duodenum just beyond the pylorus and then

* Wolff, Zeitschr. f. klin. Med., 1889, xvi, p. 222; Jahresbericht f. Thierchemie, 1889, xix, p. 266.

† Klemperer, Zeitschr. f. klin. Med., 1890, xvii, Supp., p. 324; Centralbl. f. med. Wissen., 1891, p. 751.

‡ Blumenau, Therapeutische Monatshefte, 1890, v, p. 504; Jahresbericht f. Thierchemie, 1891, xxi, p. 212.

§ Brandl, Zeitschr. f. Biologie, 1892, xxix, p. 304.

‖ Haan, Comptes rendus de la société de biologie, 1895, ii, p. 817.

introducing a definite volume of the fluid to be examined
into the empty stomach in the manner already indicated in
previous experiments. In several cases, dogs with gastric
fistulæ were employed. The abdomen was quickly sewed up
after this operation, chloroform-ether stopped, and the animal
allowed entire freedom of movement. The liquid employed
was ordinarily warmed gently to avoid the asserted stimulat-
ing action of cold fluids on the gastric mucosa.* Ligations of
the œsophagus and œsophageal fistulæ were avoided, since a
somewhat extended experience with gastric fistula dogs, as
well as the experiments about to be described, have convinced
us, in agreement with Heidenhain's observations,† that under
ordinary circumstances, *i. e.*, in the absence of unusual stimuli
(and with slightly narcotized animals) the amount of saliva
secreted is small at most, and fails to induce any pronounced
secretion in the stomach.‡ Further, we have found that
an unusual flow of saliva is at once readily detected by the
physical character of the stomach contents, *e. g.*, frothing, etc.
Furthermore, the conditions of our experiments were intended
to approach those normally obtaining in the body as nearly
as possible; and finally, a sufficient number of control experi-
ments in which water was introduced into the stomach, have
left no doubt as to the validity of the method. At the end
of three to four hours — a period shown by our experiments
to cover the digestion time of a test meal for the dog — the
animal was bled to death, the œsophagus ligated at the lower
end, the stomach removed from the body, wiped free from
blood, and the contents discharged into a graduated vessel.
In the fluid thus obtained, total acidity, free and combined
HCl, and acid reacting salts were determined by the method
of Töpfer; § alcohol was estimated, when present, in the
distillate from a definite portion of the gastric contents, by
the pycnometer method; total solids were determined by

---

* Cf. Kühne, Lehrbuch der physiol. Chemie, p. 28.

† Hermann's Handbuch, v, p. 112.

‡ Compare also the experiment described on page 76.

§ Töpfer. Zeitschr. f. physiol. Chemie, 1894, xix, p. 104.

drying a weighed quantity of fluid in a tared crucible at 100–105° C. Protocols follow : —

A. Control Experiments with Water : —

I. 31 v, 1897. Dog, with gastric fistula, well healed. Weight 21 kilos. Fluid removed completely through fistula.

Introduced 200 c. c. *distilled water* at 10.50 A. M.
Contents removed at 1.55 P. M. = $3\frac{1}{12}$ hrs.

Volume of fluid recovered from stomach = 160 c.c. = **80 per cent** of original volume.

Analysis of the contents gave :

Total acidity	0.203 per cent.*
Free HCl	0.192
Loosely combined HCl . . .	0.002
Salts	0.009
Total solids	0.624

II. 28 vi, 1897. Dog, with gastric fistula, well healed. Weight 25 kilos. Fluid removed completely through fistula.

Introduced 135 c. c. *distilled water* at 11 A. M.
Contents removed at 1.45 P. M. = $2\frac{3}{4}$ hrs.

Volume of fluid recovered from stomach = 110 c. c. = **81 per cent** of original volume.

Analysis of the contents gave :

Total acidity	0.274 per cent.
Free HCl	0.241
Loosely combined HCl . . .	0.018
Salts	0.015
Total solids	0.77

III. 24 v, 1897. Dog. Weight 7.7 kilos.

Introduced 125 c. c. *distilled water* at 10 A. M.
Contents removed at 1.50 P. M. = $3\frac{2}{3}$ hours.

Volume of fluid recovered from stomach = 114 c. c. = **91 per cent** of original volume.

Analysis of the contents gave :

Total acidity	0.094 per cent.
Free HCl	0.065
Loosely combined HCl . .	0.004
Salts	0.025
Total solids	0.47

* Expressed as HCl in all the experiments.

IV. 29 v, 1897.   Dog.   Weight 14.5 kilos.

Introduced 200 c. c. *distilled water* at 9.30 A. M.
Contents removed at 1.15 P. M. = $3\frac{3}{4}$ hrs.

Volume of fluid recovered from stomach = 206 c. c. = **103 per cent** of original volume.*

Analysis of the contents gave:

Total acidity	0.047 per cent.
Free HCl	0.040
Loosely combined HCl . .	0.004
Salts	0.003
Total solids.	0.50

V. 2 vi, 1897.   Dog.   Weight 10.5 kilos.

Introduced 125 c. c. *carbonated water* at 9 A. M.
Contents removed at 12.45 P. M. = $3\frac{3}{4}$ hrs.

Volume of fluid recovered from stomach = 125 c. c. = **100 per cent** of original volume.

Analysis of the contents gave:

Total acidity	0.191 per cent.
Free HCl	0.152
Loosely combined HCl . .	0.014
Salts	0.025
Total solids.	0.55

In this experiment the CO_2 was completely absorbed.

VI. 1 vii, 1897.   Dog.   Weight 10 kilos.

Introduced 76 c. c. of 2 per cent *dextrose* solution at 9.10 A. M.
Contents removed at 12.40 P. M. = $3\frac{1}{2}$ hrs.

Volume of fluid recovered from stomach = 68 c. c. = **90 per cent** of original volume.

Analysis of the contents gave:

Total acidity	0.072 per cent.
Free HCl	0.047
Loosely combined HCl . .	0.007
Salts	0.018

B. Experiments with strong Ethyl Alcohol : —

VII. 17 v, 1897.   Dog.   Weight 23 kilos.

Introduced 200 c. c. of 37 per cent *alcohol* at 10.45 A. M.
Contents removed at 2.15 P. M. = $3\frac{1}{2}$ hrs.

Volume of fluid recovered from stomach = 407 c. c. = **203 per cent** of original volume.

---

* A small quantity of saliva doubtless found its way into the stomach, as the dog salivated somewhat at the beginning of the operation and the stomach contents had a frothy appearance.

Analysis of the contents gave:

Total acidity	0.164 per cent.
Free HCl	0.112
Loosely combined HCl . .	0.043
Salts	0.009

VIII. 31 v, 1897. Dog. Weight 21 kilos. Gastric fistula well healed.
Contrast experiment with water and alcohol.

 a. The first part of this experiment has been described under I. p. 88.

 β. After discharge of previous stomach contents completely through fistula, 200 c. c. $37\frac{1}{2}$ per cent *alcohol* were introduced into the stomach through fistula at 1.55 P. M.

  Contents removed at 5 P. M. = $3\frac{1}{12}$ hrs.

Volume of fluid recovered from stomach = 460 c. c. = **230 per cent** of original volume.*

Analysis of the contents gave:

Total acidity	0.220 per cent.
Free HCl	0.164
Loosely combined HCl . .	0.011
Salts	0.045
Total solids	0.987

C. Experiments with weak (5 per cent) Ethyl Alcohol:—

IX. 24 vi, 1897. Bitch. Weight 8 kilos.

  Introduced 100 c. c. 5 per cent *alcohol* at 10.45 A. M.

  Contents removed at 2 P. M. = $3\frac{1}{4}$ hrs.

Volume of fluid recovered from stomach = 110 c. c. = **110 per cent** of original volume.

Analysis of the stomach contents gave:

Total acidity	0.119 per cent.
Free HCl	0.086
Loosely combined HCl . .	0.011
Salts	0.022
Total solids	0.69

X. 8 vi, 1897. Bitch. Weight 7.3 kilos.

  Introduced 110 c. c. 4.8 per cent *alcohol* at 9 A. M.

  Contents removed at 12.45 P. M. = $3\frac{3}{4}$ hrs.

Volume of fluid recovered from stomach = 135 c. c. = **123 per cent** of original volume.

Analysis of the stomach contents gave:

Total acidity	0.202 per cent.
Free HCl	0.148
Loosely combined HCl . .	0.021
Salts	0.033

---

* A post-mortem examination showed that the stomach contents could be completely discharged through the fistula by the method adopted.

The results of the foregoing experiments, expressed in percentages, are combined in the following table.

A. WITH WATER.						
	Relative Volume of Fluid at end of Experiment.	Total Acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total Solids.
I	80	0.203	0.002	0.192	0.009	0.62
II	81	0.274	0.018	0.241	0.015	0.77
III	91	0.094	0.004	0.065	0.025	0.47
IV	103	0.047	0.004	0.040	0.003	0.50
V	100	0.191	0.014	0.152	0.025	0.55
VI	90	0.072	0.007	0.047	0.018	. . .
Average.	90.8	0.147	0.008	0.123	0.016	0.58
B. WITH STRONG ALCOHOL.						
VII	203	0.164	0.043	0.112	0.009	. . .
VIII	230	0.220	0.011	0.164	0.045	0.99
Average.	216.5	0.192	0.027	0.138	0.026	0.99
C. WITH WEAK ALCOHOL.						
IX	110	0.119	0.011	0.086	0.022	0.69
X	123	0.202	0.021	0.148	0.033	. . .
Average.	116.5	0.160	0.016	0.117	0.027	0.69

A glance at the data presented leaves little doubt as to the pronounced stimulating action of pure ethyl alcohol upon gastric secretion, even with solutions of only five per cent strength. The effect is not merely one characterized by the discharge of water into the stomach cavity, but gives evidence of a true secretory process. Thus, the volume of fluid found after introduction of water into the stomach is not increased, there being rather a tendency in the opposite direction. Edkins,[*] v. Mering,[†] and others have shown that the absorption of water from the stomach is practically *nil*, while the

[*] Edkins, Journal of physiology, 1892, xiii, p. 445.

[†] v. Mering, Verhandlungen des XII. Congresses f. innere Medicin, Wiesbaden, 1893 ; Therapeutische Monatshefte, 1893, vii, p. 201.

absorption of alcohol goes on quite rapidly. In our own experiments, the alcohol used had entirely disappeared from the stomach in the course of the experiments; the question of absorption will, however, be referred to in another connection. With five per cent alcohol the increase in the volume of the gastric contents is noticeable, becoming very pronounced with the stronger percentages of alcohol. The increase in total solids gives confirmation of stimulated secretion, as does also the increase in acidity. It must be remembered, further, that the increase in acidity shown by the figures is a relative one; expressed absolutely in grams, the total acid secreted is obviously increased in far greater degree than the percentage figures indicate. The specific action of alcohol is strikingly shown in Experiment VIII, in which the conditions permitted of comparative experiments with water and alcohol on the same animal, with the following results: —

COMPARISON OF THE TWO EXPERIMENTS (VIII. a, β).

Fluid introduced into Stomach.	Fluid recovered from Stomach after 3 Hours.	Relative Volume.	Total Acidity.	Free HCl.	Loosely combined HCl.	Salts.	Total Solids.
	c. c.	per cent.					
200 c. c. water.	160	80	0.203	0.192	0.002	0.009	0.624
200 c. c. alcohol } (37½ per cent.) }	460	230	0.220	0.164	0.011	0.045	0.987

A comparison of the proteolytic activity of the two secretions by Grützner's carmine-fibrin method showed a decidedly greater digestive power in the case of the " water " secretion. Much stress cannot be placed, however, on a single experiment. The gastric fluids obtained in the experiments with alcohol possessed strong proteolytic properties in every case examined.

In view of this pronounced action of alcohol on gastric secretion it seemed desirable to ascertain something more definite regarding the way in which this process is provoked. The control experiments with water gave evidence that the mere contact of the fluid with the stomach mucosa could not

be the cause of gastric stimulation. It will be remembered that even vigorous mechanical stimulation or irritation ordinarily fails to yield more than a few grams of secretion* — an observation in decided contrast to the phenomena of gastric flow during the presence of digestible materials in the stomach. The following experiments throw light on the question raised : —

XI. 25 v, 1897. Dog. Weight 23 kilos. The intestine was ligatured just beyond the pylorus. Another ligature was applied below the point of entrance of the duct of Wirsung. 20 c. c. of 60 per cent alcohol were injected into the lumen of the intestine between these ligatures, while 105 c. c. of 60 per cent alcohol were introduced into the intestine beyond the second ligature. Then

Introduced 200 c. c. *water* into stomach at 10.45 A. M.
Contents removed at 2.30 P. M. = 3¾ hrs.

Volume of fluid recovered from stomach = 260 c. c. = **130 per cent** of original volume.

Analysis of stomach contents gave :

Total acidity . . . . . . 0.241 per cent.
Free HCl 0.213
Loosely combined HCl . . 0.002
Salts 0.026

XII. 28 v, 1897. Bitch. Weight 28 kilos. Intestine ligatured just beyond the pylorus. Another ligature was applied below the point of entrance of the duct of Wirsung. 125 c. c. of 60 per cent alcohol were injected into the lumen of the intestine below the second ligature,† then

Introduced 200 c. c. *water* into stomach at 11 A. M.
Contents removed at 2.45 P. M. = 3¾ hours.

Volume of fluid recovered from stomach = 375 c. c. = **187.5 per cent** of original volume.

Analysis of stomach contents gave :

Total acidity . . . . . . 0.333 per cent.
Free HCl 0.306
Loosely combined HCl . . 0.004
Salts 0.023
Total solids 0.30

* Cf. Tiedemann and Gmelin, Die Verdauung nach Versuchen, 1831, p. 92 ; Schiff : Leçons sur la physiologie de la digestion, ii, p. 244.

† The return of alcoholic fluid into the stomach was thus absolutely prevented.

SUMMARY OF RESULTS OF EXPERIMENTS.

No.	Relative Volume of Fluid at End of experiment.	Total Acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total Solids.
XI	130.0	0.241	0.002	0.213	0.026	. . . .
XII	187.5	0.333	0.004	0.306	0.023	0.30
Average.	158.5	0.287	0.003	0.259	0.024	0.30

From these data it seems clear that a stimulation of the gastric glands may take place, independently of any *direct* gastric irritation, in consequence of the influence of alcohol absorbed from the intestine. The volume of the fluid in the stomach increased relatively far more than when five per cent alcohol was introduced directly into the stomach (cf. Experiments IX, X, p. 90). The composition of the fluid (high acidity, free HCl, total solids) likewise gives evidence of active secretion, while the fluid was found to be strongly proteolytic. The absorption of the alcohol was complete in these experiments; and when it is remembered how quickly alcohol is distributed and disappears in the body, the actual amount reaching the gastric glands must have been relatively small, or at least must have acted during a brief period only. It seems probable, therefore, that there occurs here an indirect stimulation quite comparable to that resulting after absorption of peptone from the alimentary tract, and it is interesting to note by way of comparison that Khigine,* in his experiments upon the isolated fundus of the dog, found that the acidity of the secretion after absorption of digestion products runs parallel to a certain degree with the increase in volume. Whether the absorbed alcohol acts directly upon elements of the gastric mucosa (Heidenhain's " secondary secretion "), or becomes a stimulus to specific secretory nerve fibres (Khigine), we are unable at present to decide.†

* Khigine : Archives des sciences biologiques, St. Pétersbourg, 1895, iii, p. 461.

† Cf. Howell : American Text-book of Physiology, 1896, p. 182.

In connection with this " secondary " secretion of gastric juice due to the presence of alcohol in the small intestine, it is to be noted that Macfadyen, Nencki, and Sieber * found among the bacteria normally present in this portion of the alimentary canal species which give rise to a production of ethyl alcohol from carbohydrates ingested.

D. **Experiments with Alcoholic Beverages**. — It might naturally be assumed that the action of the various alcoholic beverages on gastric secretion would be similar, qualitatively, to that of their common constituent ethyl alcohol. Previous investigation, however, has shown that the influence of these liquors on the purely chemical processes of digestion is not necessarily proportionate to their content of alcohol, † hence it seemed desirable to study the effect of a number of typical liquors on secretion, by the method of the previous experiments. This we have done with the following results : —

XIII. 21 vi, 1897. Dog. Weight, 10.7 kilos.

> Introduced 50 c.c. **sherry** + 25 c.c. *water* (14 per cent alcohol) at 10.20 A. M.
> Contents removed at 2.15 P. M. $= 3\frac{11}{12}$ hrs.

Volume of fluid recovered from stomach = 160 c.c. = 213 per cent original volume.

> Analysis of stomach contents gave :
>
> | | |
> |---|---|
> | Total acidity | 0.367 per cent. |
> | Free HCl | 0.300 |
> | Loosely combined HCl | 0.020 |
> | Salts | 0.047 |
> | Total solids | 1.72 |

XIV. 2 vi, 1897. Dog. Weight, 18.5 kilos.

> Introduced 50 c.c. **whisky** + 100 c.c. *water* (16 per cent alcohol) at 11.15 A. M.
> Contents removed at 3 P. M. $= 3\frac{3}{4}$ hrs.

Volume of fluid recovered from stomach = 320 c.c. = 213 per cent original volume.

---

* Macfadyen, Nencki, and Sieber : **Archiv f. experimentelle Pathologie und Pharmakologie**, 1891, xxviii, p. 311.

† Chittenden and Mendel ; loc. cit.

Analysis of stomach contents gave :

Total acidity	0.382 per cent.
Free HCl	0.346
Loosely combined HCl . .	0.011
Salts	0.025
Total solids	0.42

XV. 3 vi, 1897. Bitch. Weight, 8 kilos.

Introduced 125 c.c. **Hochheimer** (13.3 per cent alcohol) at 10 A. M.

Contents removed at 1.45 P. M. = 3¾ hrs.

Volume of fluid recovered from stomach = 140 c.c. = **112 per cent** original volume.

Analysis of stomach contents gave :

Total acidity	0.230 per cent.
Free HCl	0.165
Loosely combined HCl . .	0.038
Salts	0.027

XVI. 28 vi, 1897. Dog. Weight, 25 kilos. Gastric fistula well healed.

Contrast experiment with water and wine.

α. The first part of this experiment has been described under II, p. 88.

β. After complete discharge of previous stomach contents through the fistula, 135 c.c. **white wine** were introduced into stomach through fistula at 1.45 P. M.

Contents removed at 4.30 P. M. = 2¾ hrs.

Volume of fluid recovered from stomach = 170 c.c. = **126 per cent** original volume.

Analysis of stomach contents gave :

Total acidity	0.425 per cent.
Free HCl	0.342
Loosely combined HCl . .	0.018
Salts	0.065
Total solids	1.79

XVII. 23 vi, 1897. Dog. Weight, 12.3 kilos.

Introduced 125 c.c. **claret** (5.15 per cent alcohol) at 9.30 A. M.

Contents removed at 1.30 P. M. = 4 hrs.

Volume of fluid recovered from stomach = 225 c.c. = **180 per cent** original volume.

Analysis of stomach contents gave :

Total acidity	0.378 per cent.
Free HCl	0.324
Loosely combined HCl . .	0.025
Salts	0.024
Total solids	1.90

XVIII. 18 vi, 1897. Bitch. Weight, 10.2 kilos.

Introduced 100 c.c. **lager beer** (4 to 5 per cent alcohol) at 10.20 A. M.

Contents removed at 2.15 P. M. = $3\frac{11}{12}$ hrs.

Volume of fluid recovered from stomach = 110 c.c. = **110 per cent** original volume.

Analysis of stomach contents gave :

Total acidity	0.357 per cent.
Free HCl	0.241
Loosely combined HCl . .	0.064
Salts	0.052
Total solids	9.26

XIX. 23 vi, 1897. Dog. Weight, 10 kilos.

Introduced 100 c.c. **lager beer** (4.5 per cent alcohol) at 10.10 A. M.

Contents removed at 2 P. M. = $3\frac{5}{6}$ hrs.

Volume of fluid recovered from stomach = 125 c.c. = **125 per cent** original volume.

Analysis of stomach contents gave :

Total acidity	0.241 per cent.
Free HCl	0.169
Loosely combined HCl . .	0.032
Salts	0.040
Total solids	5.51

XX. 14 vi, 1897. Dog. Weight, 14 kilos.

Introduced 150 c.c. **porter** (3.75 per cent alcohol) at 9.45 A. M.

Contents removed at 1.30 P. M. = $3\frac{3}{4}$ hrs.

Volume of fluid recovered from stomach = 195 c.c. = **127 per cent** original volume.

Analysis of stomach contents gave :

Total acidity	0.371 per cent.
Free HCl	0.320
Loosely combined HCl . .	0.036
Salts	0.015
Total solids	2.19

XXI. 7 vi, 1897. Bitch. Weight, 8.5 kilos.

Introduced 125 c.c. **lager beer** (4.7 per cent alcohol) at 10.15 A. M.

Contents removed at 2.10 P. M. = $3\frac{11}{12}$ hrs.

Volume of fluid recovered from stomach = 285 c.c. = **228 per cent** original volume.

Analysis of stomach contents gave:

Total acidity . . . . . . 0.378 per cent.
Free HCl 0.308
Loosely combined HCl . . 0.016
Salts 0.054
Total solids 2.88

XXII. 14 vi, 1897. Dog. Weight, 8.2 kilos.

Introduced 150 c.c. **porter residue** * at 11.30 A. M.
Contents removed at 3.15 P. M. = 3¾ hrs.

Volume of fluid recovered from stomach = 135 c.c. = **90 per cent**
original volume.

Analysis of stomach contents gave:

Total acidity . . . . . . 0.352 per cent.
Free HCl 0.280
Loosely combined HCl . . 0.014
Salts 0.058
Total solids 2.29

XXIII. 9 vi, 1897. Dog. Weight, 10 kilos.

Introduced 130 c.c. **lager beer residue** † at 10.30 A. M.
Contents removed at 2.30 P. M. = 4 hrs.

Volume of fluid recovered from stomach = 175 c.c. = **134 per cent**
original volume.

Analysis of stomach contents gave:

Total acidity . . . . . . 0.346 per cent.
Free HCl 0.270
Loosely combined HCl . . 0.038
Salts 0.038
Total solids 6.80

For the sake of comparison these data are contrasted in the
following table (see p. 99).

These results afford tangible evidence of the stimulating
action of the liquors examined, as shown in the increased
volume of gastric contents, accompanied by increase in acidity.
That alcohol is an important factor in the production of these
phenomena seems certain.   Contrast, for example, Experiment
XX with XXII, which differs only in the absence of the

---

* The residue left on evaporation of 150 c.c. porter, dissolved in 150 c.c.
distilled water.

† Residue from evaporation of 130 c.c. beer, dissolved in 130 c.c. water.

	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
XIV. Whisky + H_2O (16% alcohol)	218	0.382	0.011	0.346	0.025	0.42
XIII. Sherry + H_2O (13% alcohol)	213	0.367	0.020	0.300	0.047	1.72
XV. White wine (13% alcohol)	112	0.230	0.038	0.165	0.027	. . .
XVI. White wine (13% alcohol)	126	0.425	0.018	0.342	0.065	1.79
XVII. Claret (10% alcohol)	180	0.373	0.025	0.324	0.024	1.90
XVIII. Beer (4.7% alcohol)	110	0.357	0.064	0.241	0.052	9.26
XIX. Beer (4% alcohol)	125	0.241	0.032	0.169	0.040	5.51
XXI. Beer (4.7% alcohol)	228	0.378	0.016	0.308	0.054	2.88
XXIII. Residue of Beer (like XXI.)	134	0.346	0.038	0.270	0.038	6.80
XX. Porter (5.3% alcohol)	127	0.371	0.036	0.320	0.015	2.19
XXII. Residue of porter (like XX.)	90	0.352	0.014	0.280	0.058	2.29

alcohol. But the wines and malted beverages contain a variety of other constituents such as organic acids,* which perhaps contribute to increase the stimulating effect, and are doubtless partly responsible in a number of experiments for the high acidity observed. The contrast between the action of water and wine is strikingly shown in Experiments XVI, *a* and *β*, carried out on the same animal.

COMPARISON OF THE TWO EXPERIMENTS (XVI, *a*, *β*).

Fluid introduced into stomach.	Fluid removed from stomach after 3 hours.	Relative volume.	Total acidity.	Free HCl.	Loosely combined HCl.	Salts.	Total solids.
		per cent.					
135 c.c. water	110 c.c.	81	0.274	0.241	0.018	0.015	0.77
135 c.c. white wine	170 c.c.	126	0.425	0.342	0.018	0.065	1.79

* Cf. Chittenden and Mendel : loc. cit., pp. 56, 80.

The marked increase in total solids in many of these experiments, however, is not to be attributed, as it is in the case of pure alcohol, entirely to the increased secretion; it is rather in part accounted for by the unabsorbed constituents of the liquor employed. The following table, compiled from analyses at hand, shows that a large portion of the total solids in the gastric juices obtained may be derived from other sources than the secretion itself:

TABLE SHOWING TOTAL SOLIDS OF GASTRIC CONTENTS.

Nature of fluid introduced into stomach.		Total solids introduced into stomach.	Total solids in gastric contents at end of experiment.
		grm.	grm.
II.	Water . . .	0	0.84
IX.	Weak alcohol	0	0.69
VIII.	Strong alcohol	0	4.50
XIV.	Whisky . .	0.15	1.34
XVI.	White wine .	2.8	2.41
XVII.	Claret . . .	3.9	4.28
XIII.	Sherry . . .	2.35	2.78
XVIII.	Beer	7.0	10.00
XXIII.	Beer residue .	9.1	11.56
XX.	Porter . . .	6.6	4.16
XXII.	Porter residue	6.6	3.10

E. **Character of the Gastric Juice obtained by Stimulation with Alcohol.** — The gastric juice obtained as a result of the stimulating influence of alcohol and alcoholic liquors resembles that ordinarily procured from gastric fistulæ in its physical characters; it is a thin, colorless, or very faintly yellow fluid containing occasional flocks of mucus in suspension. There was no evidence of irritation or hyperæmia of the mucosa, and all traces of blood were absent. After the doses used, the gastric lining was of a pale or faintly pink color when removed after bleeding the animal. When colored alcoholic liquors were employed, the gastric contents retained the characteristic coloring matter, the latter not being absorbed, while the alcohol entirely disappeared. In chemical composition, the gastric juice appeared somewhat more acid than that ordinarily secreted. It likewise contained a larger amount of

solid matter, and in harmony with this fact the proportion of combined hydrochloric acid was increased, which in turn suggests the presence of a somewhat larger amount of proteid or other like matter. The fluids were repeatedly tested with boiled fibrin for proteolytic action, and this was always found vigorous. In the experiments in which alcohol was introduced directly into the intestine (Experiments XI, XII, p. 93), the intestinal lining was not abnormal in appearance, the reaction being alkaline to litmus in the upper duodenum and neutral or faintly alkaline further along the alimentary canal. This corresponds with the observations on the normal reaction of the intestinal contents of the dog, by Moore and Rockwood,[*] whose statements we have repeatedly verified.

GASTRIC DIGESTION.

Since chemical, mechanical, and physiological processes go on side by side during digestion, we have carried out a series of experiments to determine in what way and to what extent the factors already investigated combine or coöperate under the influence of alcohol and alcoholic liquors. Our method has included the examination of the stomach contents after test meals were given. The statements current in the literature on this subject are by no means concordant.

In experiments on a woman having a gastric fistula Kretschy [†] observed that alcohol retarded digestion. Buchner [‡] found that in the human stomach alcohol, wine, and beer all retarded digestion, though not so markedly as in artificial digestion. Bikfalvi,[§] in observations on dogs, obtained a retardation of digestion with even small quantities of alcohol. Beer and wine showed no favorable influence, the latter even retarding digestion when given in large quantities. Ogáta [||] states that

---

[*] Moore and Rockwood: Journal of Physiology, 1897, xxi, p. 373.

[†] Kretschy: Deutsches Arch. f. klin. Med., xviii, p. 527; Jahresbericht f. Thierchemie, 1876, vi, p. 173.

[‡] Buchner: Deutsches Arch. f. klin. Med., xxix, p. 537; Jahresbericht f. Thierchemie, 1881, xi, p. 286.

[§] Bikfalvi: Jahresbericht f. Thierchemie, 1885, xv, p. 273.

[||] Ogáta: Jahresbericht f. Thierchemie, 1885, xv, p. 274; Arch. f. Hygiene, 1885, iii, p. 204.

beer, wine, and brandy retard gastric digestion noticeably. Schelhaas observed * that in the living stomach wine did not retard digestion so long as there was free HCl present; pathological conditions (carcinoma ventriculi) formed the only exceptions. In an extensive series of experiments, Gluzinski † distinguishes two phases occurring during digestion in the stomach in the presence of alcohol, (1) a retardation of proteid digestion, and (2) secretion of a very active, strongly acid gastric juice. Henczinski ‡ found no bad effect on digestion following the use of beer. Blumenau § states that 25–50 per cent alcohol introduced into the healthy stomach induces a decrease in digestive action during the first two or three hours. Wolffhardt,‖ experimenting on a healthy man, concluded that 15–20 grams of absolute alcohol interfere with proteid digestion, while the effect of cognac varies with the period of digestion during which it is taken; he found that wines tend to promote digestion.

With reference to the motor functions of the stomach Lauder Brunton states that alcohol taken into this organ increases its movements as well as its secretory activity, and by mixing its contents more thoroughly with the gastric juice accelerates digestion.¶ Likewise Klemperer ** states as a result of his experiments that the motor functions are decidedly increased, as measured by the oil method, while Haan †† has more recently advanced similar conclusions as the result of work by another

---

* Schelhaas: Deutsches Arch. f. klin. Med., xxxvi, p. 427; Jahresbericht f. Thierchemie, 1885, xv, p. 271.

† Gluzinski: Deutsches Arch. f. klin. Med., 1886, xxxix, p. 405; Jahresbericht f. Thierchemie, 1886, xvi, p. 263.

‡ Henczinski: Dissertation, 1886. Quoted by Munk: Die Ernährung, p. 327.

§ Blumenau: Therapeutische Monatshefte, 1890, v, p. 504; Jahresbericht f. Thierchemie, 1891, xxi, p. 212.

‖ Wolffhardt: Münchn. med. Wochenschr., 1890, xxxvii, p. 608; Centralbl. f. med. Wissen., 1891, p. 47.

¶ Brunton: Disorders of Digestion, 1886, p. 146.

** Klemperer: Zeitschr. f. klin. Med., 1890, xvii, Supp., p. 324; Centralbl. f. med. Wissen., 1891, p. 751.

†† Haan: Comptes rendus de la société de biologie, 1895, ii, p. 816.

method. Gluzinski,[*] however, notes that alcohol diminishes the mechanical action of the stomach in moderate degree.

In considering the selection of subjects for experiment in the direction indicated, preference has been given to dogs. The series of investigations on man above referred to are already extensive, and the difficulties of obtaining definite answers to specific questions by this method of experimentation are obvious. It is rarely possible or desirable to carry out a large number of determinations on any single individual, while it is likewise practically impossible to control the physiological condition of the individual, *i. e.*, diet, etc., over prolonged periods. The animals used in this research were large dogs of 21 and 25 kilos; gastric fistulæ were made, and a German silver cannula introduced into the fundus of the stomach. In place of a cork, metal stoppers were devised to screw into the inner cannula tube by means of a small metallic key.

The arrangement is shown in the diagram. The wounds healed perfectly, and the animals remained in good health during the entire period of investigation, covering several months. Irregularities of diet were avoided by feeding definite portions of prepared dog biscuit with water; this food was eagerly eaten, and sufficed to keep the dogs in physiological equilibrium.

The determinations of the acidity of the stomach contents were carried out according to the method of Töpfer.[†] The gastric fluid was occasionally centrifugalized when food particles prevented pipetting off the fluid portion. Where only small quantities of fluid were available the titrations with phenolphthaleïn and dimethylamidoazobenzol were combined in the same 5 c.c. of fluid according to the recommendation of Einhorn.[‡] Comparative experiments show that this modifica-

---

[*] Gluzinski: loc. cit.

[†] Töpfer: Zeitschr. f. physiol. Chemie, 1894, xix, p. 104.

[‡] Einhorn: New York Medical Journal, 1896, May, 9, p. 603.

tion gives the same values as the original method. Thus in one experiment : —

	Total acidity with *Phenolphthaleïn*.	Free HCl with *Dimethylamidoazobenzol.*
Töpfer method . . . (separate titrations)	$\left\{\begin{array}{l} 1.55 \text{ c.c. } \dfrac{N}{10}\text{NaOH} \\ = 0.112 \text{ per cent HCl} . \end{array}\right.$	$\left\{\begin{array}{l} 1.0 \text{ c.c. } \dfrac{N}{10}\text{NaOH} \\ = 0.072 \text{ per cent HCl} . \end{array}\right.$
Einhorn-Töpfer method (combined titration)	$\left\{\begin{array}{l} 1.55 \text{ c.c. } \dfrac{N}{10}\text{NaOH} \\ = 0.112 \text{ per cent HCl} . \end{array}\right.$	$\left\{\begin{array}{l} 1.0 \text{ c.c. } \dfrac{N}{10}\text{NaOH} \\ = 0.072 \text{ per cent HCl} . \end{array}\right.$

Our experience with Töpfer's method (or Einhorn's modification) leads us to agree with P. Häri [*] that in the absence of free HCl., *i. e.*, when no reaction is obtained with the dimethylamidoazobenzol reagent, the quantitative determinations of HCl by this method cease to be accurate, and under such conditions it cannot be employed. The occurrence of such conditions, however, is not frequent in the dog; we have observed the absence of free HCl (during digestion) in one animal under circumstances resembling those of acute gastric catarrh.[†] The food — dog biscuit — was largely undigested many hours after the meal, the acidity was high (0.55–0.594 per cent expressed as HCl), and the gastric contents possessed an odor strongly suggesting fatty acids. Lactic acid was found present (Uffelmann's test).

In view of the increased volume of fluid found in the stomach when alcohol is introduced into that organ after ligation of the pylorus, it was of interest to learn what results follow under normal conditions of the pylorus. For this purpose 20 to 25 per cent alcohol, slightly warmed, was introduced through the gastric cannula, and at the end of 30 minutes the gastric contents were discharged into a graduated

[*] Häri, P.: Arch. f. Verdauungskrankh., ii, pp. 182, 332; Centralbl. f. Physiologie, 1896, x, p. 731.

[†] Cf. v. Jaksch: Klinische Diagnostik innerer Krankheiten, 4te Auflage, p. 200.

vessel. Control experiments were made with distilled water, both fluids always being introduced into the empty stomach. This condition of the organ is shown by the lack of spontaneous flow when the cannula is opened, as well as by absence of free HCl. Flocks of mucus, alkaline to litmus, are usually present. The data obtained show no marked agreement, the fluid as a rule rapidly disappearing from the stomach. In 17 experiments with water, the *average* relative volume recovered from the stomach through the cannula at the end of the thirty minutes after introduction of quantities from 40–200 c.c. was about 30 per cent. Fourteen similar experiments with alcohol gave an average of 45 per cent. It is natural to ascribe the relatively greater volumes found in the stomach after the use of alcohol to an increased secretion of gastric juice occurring along with the rapid expulsion of fluid through the pylorus, and not to a retardation of the motor functions; for current statements assume increased motility of the stomach under the influence of alcohol,* while the experiments already reported justify the explanation given. Much emphasis cannot, however, be placed upon the averages given above, since the individual results vary widely among themselves, and no constant corresponding variations in acidity were observed, as in the experiments with ligated pylorus.

In the following series of experiments test meals were given, and the influence of alcohol and a considerable number of alcoholic beverages contrasted with that of water. Attention was directed to (1) variations in acidity and (2) time of digestion. Fifty grams of finely chopped lean meat were fed to the dog in each experiment, the stomach having been previously examined and found empty. Meat was chosen for the test meal because experience in this laboratory has shown that its composition, when it is obtained as described, does not vary much from time to time ; and after a trial of mixed food, *e.g.* dog biscuit, it seemed more satisfactory to employ a simple diet in which proteid preponderated. Similar recommendation is made by v. Jaksch in considering test meals for the human

* Cf. references, p. 102.

subject.* Alcoholic fluids or water were introduced slightly warmed † into the stomach through the fistula, since dogs usually refuse to take the former by way of the mouth. At definite intervals of one-quarter to one-half hour, small quantities of gastric contents were permitted to flow out of the fistula. Total acidity (expressed as HCl), free and loosely combined HCl, were determined by the method already described. The process of digestion in the stomach lasted, under the conditions described, about three hours, the average duration varying somewhat with the animal.‡ There was no very gradual diminution of undissolved meat particles noticeable until toward the end of this period, when the stomach very soon became empty. This corresponds with the observations of Kühne on man and the dog, in experiments with duodenal fistulæ.§ This investigator found only a slight disappearance of contents from the stomach until near the end of the digestion period, when the great bulk of material, excepting larger pieces of food, was discharged at once through the pylorus. Richet arrived at similar conclusions in experiments on man.‖ We have usually observed a complete emptying of the stomach within a period of thirty minutes ; the conclusion of this process is designated in the notes as the " end of gastric digestion." Protocols of experiments follow.

ANALYSES OF ALCOHOLIC BEVERAGES USED.

	Alcohol by vol.	Dry solids.		Alcohol by vol.	Dry solids.
	per cent.	per cent.		per cent.	per cent.
Gin	51.0	0.29	Stout . . .	6.2	5.4
Whisky . .	50.0	0.32	Claret . . .	5.2	3.2
Sherry . . .	21.75	4.7	Porter . . .	5.3	4.4
White Wine	13.82	2.5	Beer . . .	4–5	7.0

* v. Jaksch : loc. cit., p. 192.　　　　　† Cf. note 1, p. 87.

‡ In experiments on a man, with a similar meal, Jessen found the digestion time equalled 2 to 3 hours. Zeitschr. f. Biologie, 1883, xix, p. 149.

§ Kühne : Lehrbuch der physiol. Chemie, 1868, p. 53.

‖ Richet : Quoted in Gamgee, Physiological Chemistry, 1893, ii, p. 159.

Dog A. — Weight 25 kilos.

I. 9.25 A. M. **50 grams meat (no water).**

	Total acidity.	Analysis of contents. Loosely combined HCl.	Free HCl.
9.55	0.382	0.292	0.104
10.35	0.425	0.234	0.148
11.10	0.425	0.220	0.180
11.45	0.407	0.224	0.176
12.15	Stomach empty; end of gastric digestion.		

Time of digestion = **2 hours and 55 minutes.**

II. 9.10 A. M.   50 grams meat + **50 c.c. water.**

	Total acidity.	Analysis of contents. Loosely combined HCl.	Free HCl.
9.30	0.241	0.144	0.093
10.00	0.295	0.169	0.108
10.20	0.367	0.216	0.115
10.40	0.439	0.288	0.144
11.30	Stomach empty; end of gastric digestion.		

Time of digestion = **2 hours and 20 minutes.**

III. 9.30 A. M.   50 grams meat + **100 c.c. water.**

	Total acidity.	Analysis of contents. Loosely combined HCl.	Free HCl.
10.00	0.299	0.173	0.090
10.30	0.475	0.230	0.122
11.00	0.518	0.230	0.173
11.15	0.497	0.202	0.241
11.35	0.494	0.191	0.202
11.50	0.479	0.205	0.195
12.10	0.382	0.194	0.187
12.30	Stomach empty; end of gastric digestion.		

Time of digestion = **3 hours.**

IV. 2.10 P. M.   50 grams meat + **150 c.c. water.**

	Total acidity.	Analysis of contents. Loosely combined HCl.	Free HCl.
2.40	0.252	0.137	0.108
3.10	0.374	0.194	0.130
3.40	0.533	0.245	0.198
3.55	0.547	0.234	0.234
4.10	0.490	0.205	0.216
4.25	0.385	. . .	0.101
4.40	Stomach empty; end of gastric digestion.		

Time of digestion = **2 hours and 30 minutes.**

V. 9.05 A. M.   50 grams meat + **150 c.c. carbonated water.**

	Total acidity.	Analysis of contents—Loosely combined HCl.	Free HCl.
9.35	0.263	0.083	0.122
10.05	0.360	0.158	0.140
10.35	0.468	0.194	0.216
10.50	0.486	0.205	0.216
11.05	0.540	0.234	0.198
11.25	0.580	0.234	0.248

11.45   Stomach empty; end of gastric digestion.

Time of digestion = **2 hours and 40 minutes.**

VI. 1.00 P. M.   50 grams meat + **100 c.c. 10 per cent alcohol.**

	Total acidity.	Analysis of contents—Loosely combined HCl.	Free HCl.
2.45	0.497	0.209	0.230
3.10	0.464	0.220	0.173
3.30	0.436	0.180	0.202
3.50	0.400	0.162	0.202
4.10	0.263	. . .	0.094

4.30   Stomach empty; end of gastric digestion.

Time of digestion = **3 hours and 30 minutes.**

VII. 2.30 P. M.   50 grams meat + **50 c.c. 20 per cent alcohol.**

	Total acidity.	Analysis of contents.—Loosely combined HCl	Free HCl.
3.00	0.313	0.118	0.090
3.30	0.374	0.187	0.176
4.00	0.439	0.194	0.151
4.30	0.515	0.205	0.184
5.00	0.407	0.144	0.248
5.30	0.264	. . .	0.155

5.30   Stomach nearly empty; end of gastric digestion.

Time of digestion = **3 hours.**

VIII. 12.45 P. M.   50 grams meat + **50 c.c. 20 per cent alcohol.**

	Total acidity.	Analysis of contents—Loosely combined HCl.	Free HCl.
2.30	0.439	0.213	0.158
2.50	0.457	0.191	0.205
3.10	0.493	0.205	0.227
3.30	0.364	0.129	0.187

3.50   Stomach practically empty; end of gastric digestion.

Time of digestion = **3 hours and 5 minutes.**

IX. 9.15 A. M. 50 grams meat + **50 c.c. 30 per cent alcohol.**

	Total acidity.	Analysis of contents—Loosely combined HCl.	Free HCl.
9.40	0.191	0.130	0.058
10.05	0.335	0.155	0.151
10.30	0.421	0.176	0.180
10.50	0.468	0.184	0.201
11.10	0.460	0.165	0.220
11.30	0.410	0.148	0.220
11.50	0.468	0.195	0.244
12.10	0.417	0.112	0.240
12.30	0.360	0.086	0.216

   1.00 Stomach empty ; end of gastric digestion.

Time of digestion = **3 hours and 45 minutes.**

X. 9.00 A. M. 50 grams meat + **150 c.c. Hochheimer.**

	Total acidity.	Analysis of contents—Loosely combined HCl.	Free HCl.
9.30	0.374	0.140	0.176
10.00	0.432	0.154	0.191
10.15	0.450	0.151	0.198
10.45	0.497	0.187	0.220
11.15	0.533	0.198	0.271
11.30	0.555	0.241	0.227
12.00	0.508	0.248	0.173

  12.15 Stomach empty ; end of gastric digestion.

Time of digestion = **3 hours and 15 minutes.**

XI. 9.00 A. M. 50 grams meat + **50 c.c. whisky + 50 c.c. water.**

	Total acidity.	Analysis of contents.—Loosely combined HCl.	Free HCl.
9.30	0.252	0.101	0.119
10.00	0.392	0.176	0.176
10.30	0.403	0.151	0.191

  11.00 Stomach empty ; end of gastric digestion.

Time of digestion = **2 hours.**

XII. 2.45 P. M. 50 grams meat + **50 c.c. whisky + 50 c.c. water.**

	Total acidity.	Analysis of contents—Loosely combined HCl.	Free HCl.
3.15	0.230	0.076	0.119
3.45	0.320	0.097	0.220
4.15	0.468	0.198	0.212
4.30	0.508	0.198	0.198
4.45	0.490	0.184	0.212
5.15	0.569	0.205	0.252

  5.45 Stomach empty ; end of gastric digestion.

Time of digestion = **3 hours.**

XIII. 1.00 p. m.   50 grams meat + 50 c.c. gin + 25 c.c. water.

	Total acidity.	Analysis of contents Loosely combined HCL.	Free HCL.
2.00	0.439	0.178	0.194
2.30	0.450	0.170	0.197
2.45	0.428	0.158	0.238
3.00	0.442	0.154	0.212
3.15	0.410	0.140	0.215
3.30	0.420	0.148	0.234
3.45	0.338	0.122	0.180
4.00	Stomach empty ; end of gastric digestion.		

Time of digestion = **3 hours.**

XIV. 9.20 a. m. 50 grams meat + 50 c.c. brandy + 25 c.c. water.

	Total acidity.	Analysis of contents Loosely combined HCL.	Free HCL.
9.50	0.237	0.159	0.065
10.20	0.368	0.201	0.133
10.50	0.465	0.230	0.205
11.20	0.533	0.267	0.194
11.40	0.468	. . .	0.158
12.00	Stomach empty ; end of gastric digestion.		

Time of digestion = **2 hours and 40 minutes.**

XV. 2.50 p. m.   50 grams meat + 150 c.c. lager beer.

	Total acidity.	Analysis of contents. Loosely combined HCL.	Free HCL.
3.20	0.259	0.112	0.115
3.50	0.410	0.205	0.148
4.20	0.518	0.245	0.184
4.35	0.572	0.248	0.230
4.50	0.569	0.252	0.208
5.05	0.547	0.220	0.238
5.20	0.508	0.162	0.211
5.35	0.475	0.162	0.238
5.50	0.413	0.115	0.241
6.05	Stomach empty; end of gastric digestion.		

Time of digestion = **3 hours and 15 minutes.**

XVI. 9.40 a. m.   50 grams meat + 150 c.c. stout.

	Total acidity.	Analysis of contents Loosely combined HCL.	Free HCL.
10.10	0.364	0.140	0.187
10.40	0.446	0.166	0.180
11.10	0.555	0.220	0.295
11.40	0.616	0.212	0.302
12.10	0.580	0.266	0.247
12.40	Stomach empty; end of gastric digestion.		

Time of digestion = **3 hours.**

XVII α. 9.15 A.M. 50 grams meat + 150 c.c. beer.

| | Total acidity. | Analysis of contents | |
		Loosely combined HCl.	Free HCl.
9.45	0.248	0.151	0.082
10.15	0.367	0.201	0.123
10.45	0.457	0.238	0.137
11.20	0.526	0.266	0.209
11.40	0.511	0.218	0.223
12.15	0.465	0.216	0.176

12.30 Stomach empty; end of gastric digestion.

Time of digestion = **3 hours and 15 minutes.**

XVII β. 3.00 P.M. 50 grams meat + 150 c.c. water.

| | Total acidity. | Analysis of contents | |
		Loosely combined HCl.	Free HCl.
3.30	0.227	0.130	0.090
4.00	0.400	0.209	0.129
4.30	0.522	0.274	0.158
5.00	0.583	0.310	0.195
5.15	0.583	0.302	0.205
5.30	0.446	0.209	0.184
5.45	0.569	0.298	0.127

6.00 Stomach empty; end of gastric digestion.

Time of digestion = **3 hours.**

XVIII α. 8.30 A.M. 50 grams meat + 50 c.c. water.

| | Total acidity. | Analysis of contents | |
		Loosely combined HCl.	Free HCl.
9.00	0.371	0.227	0.126
9.30	0.443	0.274	0.144
10.00	0.518	0.252	0.234
10.30	0.569	0.263	0.252

11.00 Stomach empty; end of gastric digestion.

Time of digestion = **2 hours and 30 minutes.**

XVIII β. 2.10 P.M. 50 grams meat + 100 c.c. 30 per cent alcohol.

| | Total acidity. | Analysis of contents | |
		Loosely combined HCl.	Free HCl.
2.40	0.234	0.112	0.101
3.10	0.352	0.165	0.137
3.40	0.490	0.209	0.162
4.10	0.550	0.263	0.191
4.40	0.550	0.245	0.201

5.10 Stomach empty; end of gastric digestion.

Time of digestion = **3 hours.**

XIX a. 9.00 A.M.　50 grams meat + 100 c.c. **water.**

| | | Analysis of Contents | |
	Total acidity.	Loosely combined HCl.	Free HCl.
9.30	0.324	0.165	0.137
10.00	0.378	0.198	0.144
10.30	0.494	0.259	0.169
11.00	0.487	0.220	0.188
11.15	0.457	0.205	0.131
11.30	Stomach empty; end of gastric digestion.		

Time of digestion = **2 hours and 30 minutes.**

XIX β. 2.30 P.M.　50 grams meat + 150 c.c. **lager beer.**

| | | Analysis of Contents | |
	Total acidity.	Loosely combined HCl.	Free HCl.
3.00	0.260	0.119	0.187
3.30	0.378	0.201	0.187
4.00	0.465	0.191	0.188
4.30	0.533	0.223	0.248
4.45	0.562	0.233	0.306
5.10	0.465	0.223	0.176
5.30	Stomach empty; end of gastric digestion.		

Time of digestion = **3 hours.**

XX a. 9.15 A.M.　50 grams meat + 75 c.c. **sherry** + 25 c.c. **water.**

| | | Analysis of Contents | |
	Total acidity.	Loosely combined HCl.	Free HCl.
9.45	0.295	0.108	0.155
10.15	0.331	0.101	0.173
10.45	0.367	0.138	0.187
11.15	0.418	0.158	0.212
11.30	0.436	0.169	0.216
11.45	0.490	0.191	0.248
12.00	Stomach empty; end of gastric digestion.		

Time of digestion = **2 hours and 45 minutes.**

XX β. 2.30 P.M.　50 grams meat + 150 c.c. **carbonated water.**

| | | Analysis of Contents | |
	Total acidity.	Loosely combined HCl.	Free HCl.
3.00	0.238	0.043	0.126
3.30	0.360	0.130	0.176
4.00	0.432	0.187	0.169
4.30	0.533	. . . .	0.169
4.45	Stomach empty; end of gastric digestion.		

Time of digestion = **2 hours and 15 minutes.**

Dog B. — Weight 21 kilos.

I. 1.45 P.M. **50 grams meat (no water).**

	Total acidity.	Analysis of Contents——Loosely combined HCl.	Free HCl.
2.15	0.353	0.191	0.118
2.40	0.443	0.222	0.180
3.00	0.511	0.227	0.198
3.20	0.525	0.227	0.280
3.45	0.572	0.260	0.209
4.15	0.568	0.349	0.195

4.45 Stomach empty; end of gastric digestion.

Time of digestion = **3 hours.**

II. 9.15 A.M. **50 grams meat + 50 c.c. water.**

	Total acidity.	Analysis of Contents——Loosely combined HCl.	Free HCl.
9.50	0.302	0.220	0.082
10.15	0.432	0.223	0.144
10.45	0.472	0.201	0.252
11.15	0.472	0.144	0.288
11.35	0.484	0.155	0.270
11.55	0.453	0.144	0.306
12.15	0.407	0.100	0.241
12.30	0.400	0.133	0.234
12.45	0.306	....	0.216

End of gastric digestion.

Time of digestion = **3 hours and 30 minutes.**

III. 9.15 A.M. **50 grams meat + 50 c.c. 20 per cent alcohol + water.**

	Total acidity.	Analysis of Contents——Loosely combined HCl.	Free HCl.
9.50	0.136	0.086	0.036
10.15	0.285	0.108	0.144
10.45	0.479	0.173	0.244
11.15	0.472	0.177	0.252
11.35	0.518	0.237	0.252
11.55	0.486	....	0.209
12.15	0.421	....	0.213

12.30 Stomach empty; end of gastric digestion.

Time of digestion = **3 hours and 15 minutes.**

8

IV.   8.50 A.M.   **50 grams meat + 100 c.c. 30 per cent alcohol.**

	Total acidity.	Analysis of Contents — Loosely combined HCl.	Free HCl.
9.20	0.324	. . .	0.144
9.50	0.493	. . .	0.072
10.20	0.641	. . .	0.100
10.50	0.547	0.388	0.166
11.20	0.588	. . .	0.206
11.50	0.544	. . .	0.230
12.20	. . .	. . .	present.
12.30	End of gastric digestion.		

Time of digestion = **3 hours and 40 minutes.**

V.   2.45 P.M.   **50 grams meat + 75 c.c. claret.**

	Total acidity.	Analysis of Contents — Loosely combined HCl.	Free HCl.
3.15	0.396	0.155	0.216
3.45	0.450	0.238	0.158
4.15	0.576	. . .	0.209
4.45	End of gastric digestion.		

Time of digestion = **2 hours.**

VI *a.*   9.15 A.M.   **50 grams meat + 150 c.c. beer.**

	Total acidity.	Analysis of Contents — Loosely combined HCl.	Free HCl
9.45	0.273	0.144	0.104
10.15	0.367	0.187	0.155
10.45	0.464	0.223	0.194
11.15	0.616	0.345	0.256
11.45	0.501	0.238	0.170
12.15	0.508	. . .	0.151
12.30	0.533	. . .	0.187
12.45	0.468	. . .	0.158
1.00	End of gastric digestion.		

Time of digestion = **3 hours and 45 minutes.**

VI *β.*   1.00 P.M.   **50 grams meat + 150 c.c. water.**

	Total acidity.	Analysis of Contents — Loosely combined HCl.	Free HCl.
2.00	0.620	0.282	0.201
2.30	0.590	0.266	0.234
3.00	0.666	0.392	0.224
3.30	0.627	. . .	0.206
4.00	. . .	. . .	trace.
4.15	End of gastric digestion.		

Time of digestion = **3 hours and 15 minutes.**

In the accompanying table the "time of digestion" of the experiments preceding is given in hours. The experiments marked a and β are strictly comparable, as reference to the protocols will show that they were carried out in succession on the same day.

From these results it is apparent that the time of digestion in the stomach for the proteid test meal employed is not greatly varied under the influence of alcohol. The results obtained suggest, however, a tendency toward prolongation of the period during which the meat remains in the stomach when alcoholic fluids are present. This tendency is most noticeable in the case of Dog A, and particularly in those experiments which immediately succeed each other on the same day and are therefore strictly comparable. The differences are too small, however, to have any great significance. Retardation is perhaps more marked with the malted beverages, and is apparently out of proportion to the alcohol present. With reference to the changes in the acidity of the stomach contents a large number of observations disclose no specific differences in the various digestions. The variations are common to all the experiments. They include a gradual rise in total acidity during approximately the first two hours of digestion, followed by a gradual decrease until the stomach becomes empty; at this point free HCl is absent. The combined HCl increases with the progress of digestion, the products of proteolysis combining with relatively larger quantities of free acid.* Since the secretion of acid is continually progressing in the stomach, the percentage of free HCl increases gradually in the course of the digestion, likewise decreasing rapidly toward the end of this process. In agreement with our previous statements relative to the rather sudden discharge of the gastric contents into the intestine (p. 106), an abrupt decline in acidity toward the end of the digestion period was frequently observed. Evidence of an "after period" of secretion was not obtained.†

* Cf. Chittenden, Digestive Proteolysis, 1894, pp. 53 seq.
† Cf. Gluzinski, Jahresbericht f. Thierchemie, 1886, xvi, p. 264.

TABLE OF TIME OF DIGESTION (IN HOURS).

	Dog A.					Dog B.		
No.	Water.	Alcohol	Weak Alcoholic Beverages.	Strong Alcoholic Beverages.	No.	Water.	Alcohol.	Weak Alcoholic Beverages.
	h. m.	h. m.	h. m.	h. m.		h. m.	h. m.	h. m.
XVII. {α β}	3	....	3 15	....	I.	3	....	....
I.	2 55	....	....	....	II.	3 30	....	....
II.	2 20	....	....	....	III.	....	3 15	....
VII.	....	3	....	....	IV.	....	3 40	....
VI.	....	3 30	....	....	V.	....	....	2
VIII.	....	3 05	....	....	VI. {α β}	3 15	....	3 45
IX.	....	3 45	....	....		....	....	....
XVIII. {α β}	2 30	3	....	....		....	....	....
XIV.	....	....	....	2 40		....	....	....
XV.	....	....	3 15	....		....	....	....
XIX. {α β}	2 30	....	3	....		....	....	....
XVI.	....	....	3	....		....	....	....
IV.	2 30	....	....	....		....	....	....
X.	....	....	3 15	....		....	....	....
III.	3	....	....	....		....	....	....
XIII.	....	....	....	3		....	....	....
XX. {α β}	2 15	....	....	2 45		....	....	....
V.	2 40	....	....	....		....	....	....
XI.	....	....	....	2		....	....	....
XII.	....	....	....	3		....	....	....
Average.	2 40	3 20	3 10	2 40		3 15	3 30	2 52

Disappearance of Alcohol from the Stomach.

It has long been known that alcohol disappears rapidly from the alimentary canal, and even so early as 1847 Bouchardat and Sandras stated that the absorption takes place from the stomach especially.* More recent and conclusive experiments in which the pylorus has been artificially closed, have demonstrated with certainty that alcohol, in distinction from water, is readily absorbed from the stomach.† Furthermore, many substances like sugar, peptone, etc., are readily absorbed from the stomach in the presence of alcohol, while their absorption from the intestine is likewise accelerated by this substance.‡ Thus an ordinary dose of chloral hydrate introduced in watery solution into a stomach with ligated pylorus fails to bring about narcosis; § if, however, a quantity of alcohol too small of itself to produce any pharmacological action be present, narcosis follows, just as when the open pylorus permits the intestine to participate in the absorption.

The complete disappearance of alcohol from the stomach has been observed by us in a large number of experiments in which the pylorus was ligated. The following results tabulated from the experiments on secretion (pp. 88–99), demonstrate this statement : —

The rapid discharge of watery or alcoholic fluids from the stomach through the pylorus has already been referred to on p. 105. The results are in harmony with those obtained by v. Mering on dogs with duodenal fistulæ.‖ In his experiments, for example, 500 c.c. being administered to a large dog, 490 c.c. were expelled through the pylorus in twenty minutes. The

* Bouchardat and Sandras, Annales de chimie et de physique, 1847, xxi, 3. Série, p. 456.

† Cf., for example, Tappeiner, Zeitschr. f. Biologie, 1881, xvi, p. 497 ; Brandl, ibid., 1892, xxix, p. 277 ; v. Mering, Jahresbericht f. Thierchemie, 1893, xxiii, p. 293.

‡ Cf., for example, J. v. Scanzoni, Zeitschr. f. Biologie, 1896, xxxiii, p. 462.

§ Cf. also experiments with strychnine. Meltzer, Journ. of exper. medicine, 1896, i, p. 529.

‖ v. Mering, quoted in Gamgee, Physiological chemistry, 1893, ii, pp. 441 seq.

TABLE SHOWING ABSORPTION OF ALCOHOL FROM STOMACH.

No.	Weight of Dog.	Duration of Experiment.	Volume of Fluid Introduced.	Content of Alcohol. Per cent by Volume.	Alcohol Found at end of Experiment.
	kilos.	h. m.	c.cm.		grm.
VII.	23.0	3 30	200 (alcohol)	37.5	4
VIII.	21.0	3 00	200 (alcohol)	37.5	4–5
IX.	8.0	3 50	100 (alcohol)	5.0	0
X.	7.3	3 45	110 (alcohol)	4.8	0
XIII.	10.7	3 55	75 (sherry)	21.0	0
XIV.	18.5	3 45	150 (whiskey)	16.0	0
XV.	8.0	3 45	125 (wine)	13.3	0
XVI.	25.0	3 00	135 (wine)	13.3	0
XVII.	12.3	4 00	125 (claret)	5.15	0
XVIII.	10.2	3 55	100 (beer)	4.5	0
XX.	14.0	3 45	150 (porter)	3.75	0
XXI.	8.5	3 55	125 (beer)	4.7	0

rapidity of expulsion was found to depend on the state of repletion of the small intestine, — an observation in accord with the retarded evacuation of the stomach seen when food is given along with fluids.   v. Mering further observed that when water holding CO_2 in solution enters the stomach, the gas is readily absorbed; [*] alcohol is likewise absorbed, as J. Miller has recently verified for the human stomach.[†] Ogáta [‡] found that of 6.5–8.8 grams of alcohol introduced into the stomach in wine or beer, 80–90 per cent disappeared within half an hour. In the presence of soluble products in the stomach, an excretion of water by that organ is said to result in proportion to the amount of substance absorbed, — an idea akin to the one suggested in explanation of the relatively larger quantities of fluid found in the unligated stomach soon after introduction of alcohol, as compared with water.   The experiments which we have made verify the statements of the investigators mentioned, as the following data selected from protocols indicate: —

[*] Cf. also Experiment V., p. 89.

[†] Miller, J., Arch. f. Verdauungskrankh., i, p. 233.   Jahresbericht f. Thierchemie, 1895, xxv, p. 293.

[‡] Ogáta, Jahresbericht f. Thierchemie, 1885, xv, p. 274.

DATA SHOWING DISAPPEARANCE OF ALCOHOL FROM UNLIGATED STOMACH.

I. Dog, with gastric fistula.

    a. 3.45 p. m.   Introduced *50 c.c. 20 per cent alcohol* into stomach.

       4.15 "   Removed gastric contents = *40 c.c.   No alcohol found.*

    b. 3.15 "   Introduced *40 c.c. 25 per cent alcohol.*

       3.45 "   Removed gastric contents = *20 c.c.   No alcohol found.*

    c. 2.40 "   Introduced *125 c.c. 20 per cent alcohol.*

       3.10 "   Removed a portion of gastric contents. Free HCl = 0.072 per cent. *Small amount of alcohol present.*

II. Dog of 18 kilos, employed in a salivary experiment. In the course of the latter the animal received at intervals *45 c.c. absolute alcohol* diluted with water. Two hours after last portion was given the stomach contents (200 c.c) were removed. They contained *1.1 grms. alcohol.*

III. Dog of 18 kilos. Salivary experiment. At intervals were given *70 c.c. absolute alcohol* diluted with water. One and one-third hours after last portion (40 c.c.) was given the stomach contents (350 c.c.) contained *9.4 grms. alcohol.*

IV. Dog of 14 kilos. Salivary experiment. *140 c.c. absolute alcohol* diluted with water were given in three portions. Three-fourths of an hour after the last portion (50 c.c.), the stomach contents (450 c.c.) contained *24.6 grms. alcohol.*

V. Dog of 10 kilos. Salivary experiment. *120 c.c. whiskey,* containing 50 per cent of alcohol, were given in two portions. Four and one-half hours after the last portion (60 c.c.) the stomach contents (170 c.c.), contained *2.7 grms. alcohol.*

VI. Dog. Salivary experiment. *135 c.c. brandy,* containing about 50 per cent of alcohol, were given in two portions. Two hours after last portion (75 c.c.), the stomach contents (240 c.c.) contained *8.8 grms. alcohol.*

VII. Dog of 10 kilos. Salivary experiment. *350 c.c. wine,* containing 5.15 per cent alcohol, were given in two portions. One and one-half hours after last portion (200 c.c.), the stomach contents (190 c.c.) contained *5.5 grms. alcohol.*

It is of interest to note that the large volumes of fluid (170–450 c.c.) found in the stomach in Experiments II.–VII. correspond with the data already presented with reference to the increased secretion of gastric juice due to alcohol and alcoholic beverages.

SUMMARY.

Some of the more important conclusions to be drawn from the results of the experiments reported in the preceding pages may be advantageously summarized here.

Upon the secretion of saliva, the presence of strong alcohol or an alchoholic beverage in the mouth has a direct stimu-

lating effect leading to a sudden increase in the flow of saliva. This acceleration of secretion, however, is of brief duration. The stimulating effect is manifested not only by an increase in the volume of the secretion, but also by an increase in both organic and inorganic constituents. The effect produced is in no sense peculiar to alcohol, but is common to many so-called stimulants, such as dilute acid (vinegar), ether-vapor, etc. Indeed the effect is precisely analogous to that induced by an increase in intensity of stimulation, when the salivary glands are electrically excited through their nerves.

As to the possibility of alcoholic fluids absorbed from the stomach giving rise to an indirect stimulation of salivary secretion, or exercising any appreciable influence upon the composition of the secretion, our results give a negative answer. Thus, alcoholic fluids introduced directly into the stomach (of dogs) by injection through the stomach wall, thus doing away with any local action in the mouth, produce no appreciable effect upon the rate of secretion, as induced by a constant external stimulus, of either submaxillary or sublingual saliva. Even doses of alcohol sufficient to produce prolonged narcosis when introduced in this way fail to check the flow of saliva. There is likewise no specific influence exerted on the composition of the secretion. Hence, so far as our results go, alcohol and alcoholic fluids are without any specific effect upon the secretion of saliva, except to produce a transitory stimulation of secretion while in the mouth cavity.

Upon gastric secretion, alcohol and alcoholic fluids have a marked effect, increasing very greatly both the flow of gastric juice and also its content of acid and total solids. Further, this action is exerted not only by the presence of alcoholic fluids in the stomach, but also indirectly through the influence of alcohol absorbed from the intestine. Thus ordinary ethyl alcohol introduced into the empty stomachs of dogs, with the duodenum ligated, shows a marked stimulating action upon gastric secretion — as compared with the action of water under like conditions — increasing not only the volume of

gastric juice very greatly, but also its acidity, content of solid matter, etc. Moreover, alcohol absorbed from the intestine, the latter being entirely shut off from the stomach, may likewise cause stimulation of the gastric glands, with a marked increase in the rate of secretion, etc. Whiskey, brandy, sherry, claret, beer, and porter all agree in producing stimulation of gastric secretion. Further, as already stated, the gastric juice secreted under alcoholic stimulation is more acid, contains more solid matter and more combined hydrochloric acid than the ordinary secretion. It is likewise strongly proteolytic.

If these results are considered in connection with our previous observations upon the influence of alcohol and alcoholic drinks upon the purely chemical processes of gastric digestion, it is seen that side by side with the greater or lesser retardation of digestive proteolysis caused by alcoholic beverages there occurs an increased flow of gastric juice rich in acid and of unquestionable digestive power. The two effects may thus normally counterbalance each other, though it is evident that modifying conditions may readily retard or stimulate the processes in the stomach according to circumstances. Foremost among the latter is the rapid disappearance of alcohol from the alimentary canal.

Since any influence exerted by alcohol or alcoholic beverages upon the solvent or digestive power of the gastric juice in the stomach must depend upon the presence of alcohol in the stomach contents, it follows that the tendency toward rapid removal of the alcohol from the alimentary tract by absorption must necessarily diminish correspondingly the extent of the retardation of gastric digestion which the presence of alcohol in the stomach may occasion. Since, however, the stimulation of gastric secretion induced by alcohol is brought about not only by the direct action of alcohol in the stomach, but also by the indirect action of alcohol absorbed from the intestine, it follows that possible inhibition of the digestive action of the gastric juice would probably be of shorter duration than the stimulation of secretion, and that

consequently in the body alcoholic fluids would hardly lead to any retardation of gastric digestion. This point has been very carefully and thoroughly tested by numerous experiments on healthy dogs with gastric fistulæ, using proteid test meals, with the result that certainly in the stomach of dogs digestion is not retarded in any pronounced degree under the influence of alcohol or alcoholic fluids. Of hastened digestion, the results obtained give little or no suggestion, and we must therefore conclude that the two diverse factors above referred to more or less counterbalance each other so that gastric digestion in the broadest sense of the term is not markedly varied under the influence of alcohol or alcoholic fluids. This conclusion, it may be mentioned, stands in perfect harmony with the results of the investigations of Zuntz and Magnus-Levy regarding the influence of alcohol (beer) on the digestibility and utilization of food in the body. These investigators found by a series of metabolic experiments on men with diets largely made up of milk and bread, and on individuals accustomed and unaccustomed to the use of alcoholic beverages, that the latter did not in any way diminish the utilization of the food by the body.[*]

Especially worthy of note is the rapid disappearance of alcohol from the stomach and alimentary tract when alcoholic fluids are taken. As our results show, the introduction of even 200 c.c. of 37 per cent alcohol into the stomach of a dog with the duodenum ligated at the pylorus may be followed by the nearly complete disappearance of the alcohol in 3–3½ hours by absorption through the stomach walls into the blood. With the outlet from the stomach into the intestine open, the rate of absorption of alcohol is greatly increased. We may well believe, as stated by Ogáta, that, when 6–8 grams of alcohol are taken into the stomach in the form of wine or beer, 80–90 per cent of the alcohol will disappear from the alimentary tract inside of half an hour. Indeed, our own experiments on dogs with gastric fistulæ lead to this con-

---

[*] Zuntz and Magnus-Levy, Archiv f. d. ges. Physiol., 1891, xlix, p. 438; Magnus-Levy, ibid., 1893, liii, p. 544.

clusion. Thus, in one experiment 50 c.c. of 20 per cent alcohol were introduced into the stomach, and on withdrawing the stomach-contents half an hour later no alcohol whatever was found in the 40 c.c. of fluid obtained. In view of this rapid disappearance of alcohol from the alimentary tract it is plain that alcoholic fluids cannot have much, if any, direct influence upon the secretion of either pancreatic or intestinal juice.

THE ROTARY PROPERTIES OF SOME VEGETABLE PROTEIDS.*

By ARTHUR C. ALEXANDER.

I. INTRODUCTORY.

So far as the writer has been able to ascertain, no reliable determinations have ever been made of the rotary properties of any of the vegetable proteids, although some of them can now be obtained in a pure state and in considerable quantities. The object of this investigation was to determine accurately the rotary power of some of the crystallized vegetable proteids. The globulin found in hemp seed was chosen as the main subject of study, because it could be obtained in a perfectly crystalline form with comparative ease. For purposes of comparison, the rotary powers of the crystallized globulins obtained from flaxseed and the Brazil nut were also determined under similar conditions. The identity of each of these three proteids has been completely established by the researches of Osborne,† Chittenden and Mendel,‡ and others, and a guarantee of chemical purity is furnished in their crystalline form. Their individual compositions and properties will be described later in connection with the results of the polarimeter observations.

A complete determination of the rotary properties of any substance should include a study of the effect on its rotary power of three factors — (1) the solvent used; (2) the concentration of the solution; (3) its temperature. The proteids investigated by the writer were imperfectly soluble in most solvents at the temperature of the room, and their solutions

were more or less colored. On account of their opacity, only dilute solutions containing about one or two per cent of proteid matter could be used, so that the angles measured had to be multiplied by a large reduction factor to find the rotation for the standard length and density. The errors of observation were thus multiplied many times. For this reason the results were not sufficiently exact, nor were the variations sufficiently great, to determine the manner in which the rotary power varied either with the temperature or the concentration of the solutions. The maximum range in concentration was from 0.50 per cent of proteid to about 2.50 per cent, and in temperature from 20° C. (below which precipitation was likely to occur) to 70° C., above which coagulation soon took place. The specific rotation, as will be seen later, appeared to decrease with the percentage of proteid in solution. The variation with the temperature was apparently slight, and it seemed useless to try to determine it exactly. In fact, the variation of the specific rotation with the solvent was the only factor that could be determined with any exactness.

Preparation of Solutions.

Salt Solutions. — The salt solutions used as solvents were all prepared in the same manner. A given quantity of the chemically pure salt, dried in a desiccator, was weighed out, dissolved in distilled water, and made up with distilled water to the required volume. The solution was then filtered. The residue of salt, when dried at 110° C., was next carefully determined, several portions of the solution of 20 or 30 cubic centimeters each being dried to constant weight at 110° C., and the results, which never differed by more than 2 or 3 milligrams, averaged. None of the vegetable globulins experimented upon being completely soluble in these solutions, as concentrated a solution of globulin as possible was made and then filtered clear of the undissolved proteid. The percentage of proteid matter in solution was afterward determined by drying down 20 cubic centimeters or so to constant weight at 110° C., and subtracting from the residue the weight of the

included salt. The percentage of ash in the dried proteid having been previously determined by ignition, the weight of ash- and water-free proteid in 100 cubic centimeters could easily be calculated. The concentration of the proteid solution was varied by diluting it in a graduated flask with the original solvent.

Acid and Alkaline Solutions. — The globulins studied, except when very dry, can be completely dissolved in dilute acid or alkaline solutions with comparative ease. This simplified very much the preparation of such solutions. The percentage of moisture in the globulin, which was kept in a bottle under fairly constant conditions of temperature and humidity, was carefully determined by drying it at 110° C. Thus, the percentage of moisture and of ash being known, the amount of water- and ash-free matter in a given weight could be readily found. In making the solutions a few grams of the globulin were weighed out and completely dissolved in the acid or alkali, and the solutions were then made up to the required strength in a graduated flask.

A few alkaline solutions were also prepared by the addition of a definite volume of 8 per cent potassium hydroxide to a sodium-chloride solution of the globulin. If the alkali is not too weak it can be added to a salt solution in this way without precipitating the globulin. Acids, on the contrary, produce precipitation immediately when added to such a globulin solution.

The 0.2 per cent sodium-carbonate solution used as a solvent was made in a similar manner to the neutral salt solutions, the sodium carbonate being weighed out and dissolved in distilled water. The other alkalis and acids used as solvents were made up approximately to the requisite strength with distilled water, and their exact strength was afterward determined by titration.

Apparatus and Methods of Observation.

The polarimeter observations were made in a large dark room partitioned off in the centre of the building, and a "half-

shadow " polarimeter of the Laurent type,* constructed by J. Dubose, of Paris, was employed to measure the rotary power of the proteid solutions. This was a large instrument designed for use as a saccharimeter and capable of holding tubes 400 and 500 millimeters long, with a 20-centimeter circle divided to half-degrees, and a vernier with magnifying glass reading to 0.05°. Sodium light was alone used, as no other monochromatic light could be found of sufficient intensity. The exact position of the analyzer when the two halves of the field became of the same shade could not be determined by a single setting closer than 0.05° to 0.10°. However, by taking a large number of observations and averaging them, the error of the mean could be reduced to less than 0.01°. In general, from twenty to forty settings were made and readings taken, the position of uniform shade being approached alternately from the right and the left hand. The temperature of the solution in the tube was found by removing the cover and inserting a thermometer immediately after the rotation had been measured. Instead of depending on the result of one such measurement of the rotation, the tube was always emptied and refilled with a fresh portion of the solution and a new series of readings were taken. This was repeated a number of times, and the weighted mean of the results obtained was adopted as the best value.

The specific rotation was calculated by the formula

$$(a)_D = \frac{100}{l\,c}\,a,$$

where a is the angle of rotation measured, l the length of the tube in decimeters, and c the weight of ash- and water-free substance in 100 cubic centimeters. The factor $\frac{100}{l\,c}$ in this investigation varied, according to the percentage of proteid in the solution, from 50 to as large a value as 200. Thus an error of 0.01° in measuring the angle of rotation, a, meant an error of from 0.5° to 2.0° in the value of the specific rotation, $(a)_D$.

* Dingl. Polyt. Journ., vol. ccxxiii, p. 608.

Sources of Error.

A careful investigation of all possible sources of error, both in determining the strength of the solutions and in measuring their rotary power, led to the conclusion that by far the most important source of error lay in the observer himself. The values found at different times for a single setting of the polarimeter, each the mean of thirty or forty readings and having a probable error of less than 0.01°, would frequently differ among themselves by 0.05° or more, especially when the intensity of the sodium flame varied or the observer's eyes became fatigued. The error thus introduced into the value of the specific rotation was from 2 to 5 per cent, according to the size of the angle measured, while the errors introduced by the methods of determining the percentage of proteid matter in solution, which ranked next in magnitude, never exceeded 0.3 or 0.4 per cent.

To find the probable error of the measured angle of rotation, the probable error of the initial setting with an empty tube was calculated from a large number of readings covering an interval of some days, and the probable error of the mean rotation reading was calculated in each case from the results of a number of independent series of readings. The square root of the sum of the squares of these gives the probable error in the value of the total rotation, and the probable error of the specific rotation is easily calculated from this. In calculating the probable error of a number of measurements of the same angle the abbreviated formula*

$$r_o = \frac{0.8453 \, \Sigma v}{n \sqrt{n-1}}$$

was used, where r_o is the probable error of the mean, v the deviation of each observation from the mean, Σ the usual sign of summation, and n the number of observations.

* See Merriam's Text of Least Squares, 2d ed., p. 93.

II. HEMP-SEED GLOBULIN.

Preparation and Composition.

The globulin from hemp seed can be easily obtained in a crystalline form in comparatively large quantities. The following method of preparation was employed by the writer:

The raw, ground hemp seed was treated with a 5 per cent sodium-chloride solution at 60° C. for an hour, which was then strained and filtered while hot. The filtrate obtained was rich in globulin, which was precipitated by cooling in the form of perfect but minute crystals. After decanting the supernatant liquid, the precipitate was thrown on a filter and washed successively with distilled water, dilute alcohol, absolute alcohol, and ether, thus removing all salts, fats, and other impurities soluble in water, alcohol, or ether. The ether was evaporated by exposure to the air, and the globulin was finally dried in a desiccator over sulphuric acid. In extracting the globulin in this way, about 50 grams were obtained on an average from a kilogram of the ground hemp seed. The crystals examined under a microscope and by polarized light were found to be perfect isometric octahedra with perhaps a few hexagonal plates.

The reactions of this proteid are given in full by Osborne, Chittenden and Mendel, and others.* Its composition has also been carefully determined. Chittenden and Mendel give the following analysis:

Carbon	51.63	Sulphur	0.90
Hydrogen	6.90	Oxygen	21.79
Nitrogen	18.78		100.00

0.8189 gram of the crystallized globulin prepared by the writer, dried to constant weight at 110° C., yielded, after ignition, a residue of 0.0016 gram of ash. This gives: Percentage ash = 0.20 per cent.

---

* Osborne, American Chemical Journal, vol. xiv, p. 673. Chittenden and Mendel, Journal of Physiology, vol. xvii, p. 50.

Sodium-chloride Solutions.

The mode of preparing these solutions has already been described. Hemp-seed globulin is fairly soluble in a 10 per cent sodium-chloride solution at temperatures above 20° C. At 15° C. it is much less soluble, and at 10° C. it is only very slightly soluble. The solutions of hemp-seed globulin are of a brownish-yellow color. Although somewhat opaque, the writer was able to use solutions containing as much as 2 or 3 per cent of the proteid in a 100 millimeter tube.

Four independent solutions of hemp-seed globulin were made in a 10 per cent sodium-chloride solution, and the percentage of globulin in solution was determined by drying at 110° C. The results of the polarimeter observations with these solutions are shown in the following table:

No. of Solution.	Ash-free Glob. in 100 c.c.	Tem-pera-ture.	No. of Meas-urements.	Length of Tubes.	Mean Rota-tion per 100 mm.	Mean Value $(a)_D$.	Prob-able Error.	Remarks.
	grm.	deg.C.		mm.	deg.	deg.	deg.	
1	3.40	25.0	2	100	−1.47	−43.2	±0.5	} Solution quite
2	2.91	26.5	3	100	1.26	43.2	1.0	} opaque.
4	2.30	24.8	4	100	0.995	43.2	0.5	Less opaque.
1 (diluted)	1.70	24.8	6	100 and 200	0.730	42.9	0.8	
2 (diluted)	1.45	26.4	2	100	0.596	41.0	2.0	Eyes fatigued.
2 (diluted)	0.97	26.2	4	100 and 200	0.389	40.0	1.1	
4 (diluted)	0.92	20.3	6	200	0.365	39.7	0.9	
3	0.80	22.3	9	200 and 300	0.342	43.0	0.9	

With the exception of those for solutions containing 0.97 and 0.92 per cent of globulin, the values obtained for $(a)_D$ agree well within the given limits of error. In general they seem to indicate a decrease in the rotary power of the globulin with the percentage in solution. Assuming such a decrease in the rotary power, by interpolating and extrapolating for each separate solution and averaging the results we obtain $(a)_D = −41.6° \pm 0.5°$ as the specific rotation of hemp-seed globulin dissolved in a 10 per cent sodium-chloride solution, each 100 cubic centimeters of the resultant solution

containing one gram of the proteid. For purposes of comparison, the value

$$(a)_D = -41.5°$$

probably represents the specific rotation of hemp-seed globulin in such a solution within at least 1°.

Sodium-sulphate Solutions.

The sodium-sulphate solution used as a solvent was made up to a strength of 10 per cent with sodium sulphate dried in a desiccator, but of only 5 per cent of the anhydrous salt (dried at 110° C.). The hemp-seed globulin was only slightly soluble in this solution at the temperature of the room, and so the globulin solutions were made at a temperature of about 35° C. To avoid precipitation during the polarimeter observations, the tubes were warmed to about 50° C. and the solution to 35° or 40° C. before filling. Despite the care taken, several sets of observations were spoiled by the precipitation of the globulin.

Only two independent solutions of hemp-seed globulin were made in the sodium-sulphate solution. The results of the polarimeter observations and from drying down at 110° C. were as follows:

No. of solution.	Ash-free Globulin in 100 c.c.	Temperature.	No. of Measurements.	Length of Tubes.	Mean Rot. per 100 mm.	Mean Value $(a)_D$.	Probable Error.
	grm.	deg. C.		mm.	deg.	deg.	deg.
5	1.015	31.8	7	100 and 200	−0.393	−38.7	±0.8
6	0.864	30.0	5	100	0.333	38.6	1.4

These results are quite concordant, and show the specific rotation of hemp-seed globulin in a 10 per cent (5 per cent solution of the anhydrous salt) sodium-sulphate solution to be some three degrees less than in a 10 per cent sodium-chloride solution.

Ammonium-sulphate Solutions.

The hemp-seed globulin was more soluble in a 10 per cent ammonium-sulphate solution than in the preceding sodium-sulphate solution, but less soluble than in a 10 per cent sodium-chloride solution.

Two independent solutions of the globulin in a 10 per cent ammonium-sulphate solution were made, and the following results obtained from them:

No. of Solution.	Ash-free Globulin in 100 c.c.	Temperature.	No. of Measurements.	Length of Tubes.	Mean Rotation per 100 mm.	Mean Value $(a)_D$.	Probable Error.
	grm.	deg. C.		mm.	deg.	deg.	deg.
7	1.10	27.5	4	100 and 200	−0.442	−40.0	±0.8
7 (diluted)	0.48	26.5	3	200 and 220	0.205	42.8	5.6
8	1.18	30.4	6	100 and 200	0.456	38.5	1.3

These results, although not very concordant or reliable, are sufficiently so to show that the specific rotation of hemp-seed globulin in an ammonium-sulphate solution lies between the values found respectively for sodium-sulphate and sodium-chloride solutions.

The weighted mean of the above gives for the specific rotation of hemp-seed globulin in a 10 per cent ammonium-sulphate solution,

$$(a)_D = -39.8°.$$

Sodium-chloride-potassium-hydroxide Solution.

The remnants of Solutions 3 and 4 (hemp-seed globulin in a 10 per cent sodium-chloride solution) were used to form an alkaline solution by adding to them a definite volume of an 8.2 per cent potassium-hydroxide solution; 100 cubic centimeters of the solution thus formed contained 0.766 gram of hemp-seed globulin, 9 grams of sodium chloride, and 0.82 gram of potassium hydroxide.

Five measurements of the rotation due to this solution gave a mean angle of −0.371° ± 0.011° for a 100-millimeter tube, the average temperature of the solution being 22.1° C.   The

specific rotation of the hemp-seed globulin calculated from this is

$$(a)_D = -57.6° \pm 1.4°.$$

After these measurements the solution was heated to 40° C. for fifteen or twenty minutes to insure the complete conversion of the proteid into an alkali-albumin, and, after cooling, its rotary power was again determined.

Four sets of observations with a 100-millimeter tube gave a mean rotation of $-0.412 \pm 0.011°$, the average temperature of the solution being 21.2° C. The specific rotation of the globulin calculated from this

$$(a)_D = -61.6° \pm 1.4°.$$

This is only 4° greater than the value obtained before warming the solution, which would seemingly indicate that the proteid must have been almost entirely converted into an alkali-albumin before the first determination of the specific rotation was made.

Dilute Potassium-hydroxide Solution.

Two determinations of the amount of moisture in the air-dry hemp-seed globulin were made at different times by drying it at 110° C. The first determination gave a loss of weight of 0.0654 gram for each gram of globulin, and the second of 0.0659 gram, showing that the percentage of moisture remained practically unchanged.

3.3720 grams of this air-dry globulin were dissolved in an 0.18 per cent KOH solution, and made up with the same solution to 200 cubic centimeters. The rotary power of this solution of hemp-seed globulin was determined immediately after making it, and again after heating it at 40° or 50° C. for some thirty to forty minutes, with the following results:

	Ash- and Water-free Globulin in 100 c.c.	Temperature.	No. of Measurements.	Length of Tube.	Mean Rotation per 100 mm.	Mean Value $(a)_D$.	Probable Error.
	grm.	deg. C.		mm.	deg.	deg.	deg.
Before heating.	1.57	23	4	100	−1.017	−64.7	±1.0
After heating.	1.57	25	5	100	0.999	63.6	0.7

The values found for $(a)_D$ agree within the limit of error of observation, and are slightly larger than those obtained for hemp-seed globulin in the last solution. Their agreement would indicate that the action of the alkali in raising the rotary power of the globulin is exerted at once and without the aid of heat, the proteid being presumably changed more or less completely into an alkali-albumin when dissolved in the alkaline solution.

Dilute Sodium-carbonate Solution.

5.6987 grams of the air-dry hemp-seed globulin were dissolved in a 0.2 per cent sodium-carbonate solution and then made up with the same sodium-carbonate solution to 240 cubic centimeters. The rotary power of this globulin solution was determined, (1) immediately after making up the solution, (2) six hours after, and (3) after heating it at 40° to 50° C. thirty or forty minutes, and cooling. The results of the polarimeter observations were as follows:

	Ash- and Water-free Globulins in 100 c.c.	Temperature.	No. of Measurements.	Length of Tubes.	Mean Rotation per 100 mm.	Mean Value $(a)_D$.	Probable Error.
	grm.	deg. C.		mm.	deg.	deg.	deg.
1	2.21	22.0	4	100	−1.072	−48.4	±0.5
2	2.21	22.5	4	100	1.105	49.9	0.4
3	2.21	23.0	4	100	1.200	54.2	0.7

These show a slight increase in the specific rotation during the first six hours, and a marked increase after heating the solution of over 4°. The globulin is evidently not so readily nor so completely transformed by the sodium carbonate as by the stronger caustic alkali.

Dilute Hydrochloric-acid Solution.

3.6906 grams of the air-dry hemp-seed globulin were dissolved in a 0.26 per cent hydrochloric-acid solution and made up to 262 cubic centimeters with the same solution. This solution was exceptionally transparent and the most satisfac-

tory for use in the polarimeter of any proteid solution tried by the writer. Its rotary power was determined, (1) two hours after making the solution up, (2) twenty hours after making it up, (3) after heating it at about 40° C. for twenty minutes, and (4) after letting it stand for thirty-four days. The results of the polarimeter observations were as follows:

	Ash- and Water-free Globulins in 100 c.c.	Temperature.	No. of Measurements.	Length of Tubes.	Mean rot. per 100 mm.	Mean Value $(a)_D$.	Probable Error.
	grm.	deg. C.		mm.	deg.	deg.	deg.
1	1.31	24	6	100	−1.120	−85.3	±0.7
2	1.31	20	4	200	1.066	81.2	0.4
3	1.31	21	4	300	1.079	81.9	0.2
4	1.31	24	4	200	1.097	83.6	0.3

The largest value obtained for the specific rotation was within two hours after making the solution up and before heating it. That obtained twenty hours after was some 4° lower, and there was only a slight increase in the specific rotation after the solution had been heated. After the solution had stood for over a month the specific rotation was found to have increased about 1.7°.

III. FLAX-SEED GLOBULIN.

Preparation and Composition.

On account of the gummy character of the extracts from flax-seed, and the consequent difficulty in filtering them, it was not easy to obtain this proteid in large quantities. That used in this investigation was prepared by the following method:

Ordinary flax-seed meal was treated with benzine (petroleum ether) to extract the fat, and then, after drying, was passed through a fine sieve to remove the husk as far as possible. The globulin was extracted from this prepared meal by means of a 10 per cent sodium-chloride solution and the extract filtered clear. When extracted at 60° C. the extract obtained was richer in globulin but more difficult to filter than when extracted at 20° C. Extracts were obtained at both tempera-

tures. The precipitation of the globulin by cooling was found to be impracticable, even after excessive dilution of the filtrate with water. The extracts were therefore submitted to dialysis for a few days, yielding in every case a fair percentage of well-crystallized globulin. The crystallized precipitate was collected on a filter and washed successively with dilute alcohol, absolute alcohol, and ether, and dried, after the ether had evaporated, in a desiccator over sulphuric acid. The minute crystals thus obtained were perfect isometric octahedra — somewhat smaller than those obtained from the hemp-seed.

The reactions of this globulin from flax-seed have been fully given by Osborne,[*] and differ but little from those found for the hemp-seed globulin. As the result of a number of independent analyses, he found the flax-seed globulin to have the following composition:

Carbon	51.48	Sulphur.	0.81
Hydrogen	6.94	Oxygen.	22.17
Nitrogen	18.60		100.00

It will be seen by comparison that the composition of this proteid is almost identical with that of the globulin from hemp-seed, which it also resembles, as stated, in its reactions, and in the form of its crystals, except that none of the crystals, of the flax-seed globulin are of a hexagonal form. Osborne, because of the similarity in their composition and behavior toward reagents, considers these two globulins — and also those from the castor bean, cotton-seed, squash seed, and a number of cereals — as one and the same proteid body.[†] This body he has named *edestin*, from its occurrence in so many food-stuffs.

0.7778 gram of the flax-seed globulin prepared by the writer, dried at 110° C., yielded after ignition, a residue of 0.0075 gram of ash. This would give a percentage of ash of 0.96 per cent.

---

[*] American Chemical Journal, vol. xiv, pp. 629, 681.

[†] Ibid., vol. xiv, p. 687, and vol. xv, p. 24. Also Rep. Conn. Agric. Exp. Sta. for 1893, pp. 179, 216, and same for 1895, p. 172.

As the quantity of crystallized flax-seed globulin prepared was not sufficient for an extended investigation of its rotary properties, the specific rotation was determined for only a single solvent, a 10 per cent sodium chloride solution being chosen as the most available. A good basis was thus obtained for comparing its rotary power with that of the other proteids studied.

Sodium-chloride Solutions.

These solutions of flax-seed globulin had a rather strong yellow color. They proved, however, to be fairly transparent to sodium light, so that the writer was able to use in the polarimeter solutions that appeared quite opaque when in a beaker.

Three independent solutions of flax-seed globulin in 10 per cent sodium chloride were made, and used in the polarimeter. The crystals of the globulin used in the first solution (Solution 13) were somewhat imperfect, having been rounded and broken during the processes of filtering and drying. They were separated by dialysis from a 10 per cent sodium-chloride extract obtained from the fat-free flax-seed meal by a second extraction at 20° C.* The globulin used in the other two solutions contained none but perfect crystals. Part of it was extracted from the flax-seed meal by a 10 per cent sodium-chloride solution at 20° C., and the rest was similarly extracted at 60° C. In both cases the globulin was precipitated from the extract by dialysis.

The results of the polarimeter observations and from drying down are tabulated below. With the exception of the first and last, the values found for the specific rotation agree well within the limits of error, although they apparently decrease with the percentage of globulin in solution. The table shows clearly how the error in the mean value of $(a)_D$ is increased when a weak solution is used:

---

* With these were mixed some perfect crystals obtained by cooling from a 5 per cent sodium chloride extract at 60° C.

No. of Solution.	Ash-free Globulin in 100 c.c.	Temperature.	No. of Measurements.	Length of Tubes.	Mean Rotation per 100 mm.	$(a)_D$.	Probable Error.
	grm.	deg. C.		mm.	deg.	deg.	deg.
15	1.51	23.7	8	100	−0.572	−38.0	±0.5
13	1.19	25.5	10	100 and 200	0.472	39.7	0.6
14	1.08	24.0	7	100	0.426	39.4	0.9
15 (diluted)	0.94	22.5	8	200 and 300	0.364	38.8	1.2
13 (diluted)	0.60	23.0	5	200 and 300	0.225	37.8	1.8
14 (diluted)	0.47	23.5	10	200 and 300	0.172	36.7	1.3

The weighted mean of the last column but one gives

$$(a)_D = -38.7°,$$

with a probable error of less than half a degree.

Sodium-chloride-potassium-hydroxide Solution.

A remnant of Solution 15 (diluted), containing 90 cubic centimeters, was made up in a graduated flask to 100 cubic centimeters with an 8 per cent potassium-hydroxide solution. The solution thus formed contained, in 100 cubic centimeters, 0.85 gram of flax-seed globulin, 9 grams of sodium chloride, and 0.8 gram of potassium hydroxide.

The color of the solution was changed by the potassium hydroxide from a bright yellow to an orange tint, which made it more difficult to measure the rotation, even with a 100-millimeter tube. As a result of four measurements with a 100-millimeter tube at the temperature of 23° C., the rotation of the plane of polarization was found to be −0.464° ± 0.013. This would give as the specific rotation of flax-seed globulin in such an alkaline solution,

$$(a)_D = -54.5° \pm 1.5°$$

IV. Brazil-nut Globulin.

Preparation and Composition.

The globulin obtained from the Brazil nut was chosen for investigation because it differs somewhat in its chemical composition and reactions from the two previously studied. These differences are regarded by Osborne as sufficient to warrant its being classed as a distinct proteid body.* The preparations used in this investigation were obtained by the following methods:

A fine white meal was prepared from the meat of the Brazil nut by grating it, extracting the oil with benzine, and then sifting it through a fine sieve. Two methods were employed to obtain the globulin. By the first it was extracted from the prepared meal with distilled water at 60° C. and then precipitated by cooling. This precipitate was collected on a filter and washed successively with dilute alcohol, absolute alcohol, and ether, and dried, after the evaporation of the ether, over sulphuric acid. By the second method the globulin was extracted from the prepared meal with a 10 per cent sodium-chloride solution at 20° C. and the extract submitted to dialysis for a few days. The resulting precipitate was collected on a filter and washed and dried in the same manner as that obtained by cooling from the water extract. The second method gave the largest yield of globulin, and was on the whole found to be the more satisfactory, although the preparation was not as well crystallized as that from the water extract. In neither case were the globulin crystals as perfect as those obtained from the flax-seed and hemp-seed. The Brazil-nut globulin crystals varied in form from a thin hexagonal plate, through more or less rounded forms, to a perfect spheroid. The most common form, and in fact the only one found in the globulin precipitated by cooling, was a thick hexagonal plate with every other angle rounded off. In bulk the crystallized globulin appeared as a very fine, pure white powder.

* American Chemical Journal, vol. xiv, p. 687.

This globulin from the Brazil nut has been analyzed by Sacchse, Ritthausen, and others.*   The following analyses are given by Osborne:

	Crystals.	Spheroids.	Spheroids.
Carbon . . .	52.18	52.35	52.16
Hydrogen . .	6.92	6.96	6.98
Nitrogen . .	18.30	18.16	18.32
Sulphur . . .	1.06	1.12	1.07
Oxygen . . .	21.54	21.41	21.47
Totals . . .	100.00	100.00	100.00

The writer found the percentage of ash to be 0.42 per cent in the globulin preparation obtained by cooling a hot aqueous extract of the Brazil-nut meal.

Sodium-chloride Solutions.

The globulin from the Brazil nut was quite soluble in a 10 per cent sodium-chloride solution, forming a rather dense opaque fluid — so opaque to sodium light that it was difficult to measure its specific rotation, even with solutions containing from 0.5 to 1.0 per cent of the proteid.   The opacity of its solutions and the limited quantities in which this globulin could be prepared prevented an extensive investigation of its rotary properties.   As with the flax-seed globulin, the specific rotation of the Brazil-nut globulin was only determined for solutions with sodium chloride.

Three solutions of this globulin in a 10 per cent sodium-chloride solution were prepared.   The crystallized globulin used in solutions numbered 17 and 19 was obtained by cooling a hot (60° C.) water extract of fat-free Brazil-nut meal. That used in Solution 18 was obtained by dialysis from a cold (20° C.) 10 per cent sodium-chloride extract of the Brazil-nut meal.   This last solution was of a whitish color and much more opaque to sodium light than the others.   After repeated dilution a measurement of its rotary power was

* American Chemical Journal, vol. xiv, p. 669.

finally effected, but the result was so poor that no attempt was made to determine the small correction for the ash present.

The results of drying the solutions down at 110° C. and of the polarimeter observations are shown in the following table :

No. of Solution.	Globulin in 100 c.c.	Temperature.	No. of Measurements.	Length of Tube.	Mean Rot. per 100 mm.	$(a)_D$.	Probable Error.	Remarks.
	grm.	deg.C.		mm.	deg.	deg.	deg.	
17	2.13	22.3	8	100	−0.866	−40.75	±0.4	
17 (diluted)	0.92	20.8	12	100 and 200	0.366	39.9	1.9	
19	0.92	23.3	6	100	0.390	42.5	1.3	
18	0.77	22.3	2	100	0.285	37.1	2.4	Not corrected for ash.
17 (rediluted)	0.55	20.3	7	200 and 300	0.184	33.5	1.5	Unreliable ; trace of ether.
19 (diluted)	0.45	22.7	9	200	0.173	38.1	0.9	

The values found for $(a)_D$ are not very concordant or accurate, but are the best that the writer could obtain with such opaque solutions. Omitting the value −33.5°, which was somewhat doubtful, and the value 37.1°, which was not corrected for ash, the weighted mean of these is

$$(a)_D = -40.3°.$$

Sodium-chloride-potassium-hydroxide Solution. — When potassium hydroxide was added to a sodium-chloride solution of Brazil-nut globulin the resulting solution had to be made much more strongly alkaline to prevent precipitation than with solutions of either flax-seed or hemp-seed globulin.

Sixty-nine cubic centimeters of Solution 17, diluted, were made up with an 8.2 per cent potassium-hydroxide solution to 100 cubic centimeters. The resulting solution contained, in 100 cubic centimeters, 0.633 gram of ash-free globulin, 6.90 grams of sodium chloride, and 2.55 grams of potassium hydroxide.

The rotary power of this solution was determined within three hours after making up, and again after heating to 50° C. for twenty minutes, with the following results:

	Globulin in 100 c. c.	No. of Measurements.	Length of Tube.	Mean Rotation per 100 mm.	$(a)_D$.	Probable Error.	
	grm.		mm.	deg.	deg.	deg.	
Before heating	0.63	5	100	−0.370	−58.5	±1.2	} Temperature
After heating	0.63	5	100	0.364	57.5	1.3	} about 20° C.

The value of $(a)_D$ obtained after warming the solution agrees, within the limits of error, with that obtained before warming it. It is therefore obvious that the full action of the alkali is exerted without the aid of heat.*

V. SUMMARY AND CONCLUSIONS.

The specific rotation obtained from 10 per cent sodium-chloride solutions, containing about 1 per cent of proteid matter, were as follows:

Hemp-seed globulin . . . . $(a)_D = -41.5°$
Brazil-nut globulin . . . . $(a)_D = -40.5°$
Flax-seed globulin . . . . . $(a)_D = -38.5°$

The differences in the rotary powers of these substances are not very large or unexpected when we consider the slight differences in their chemical composition and behavior, and in their content of ash.

Effect of Dilution.—The observations made with sodium-chloride solutions of these globulins showed in general a decrease in the rotary power with the percentage of proteid in solution, although the observations were not sufficiently accurate to determine the rate of this decrease.

Effect of Temperature.—As already stated, the changes in the specific rotation with the temperature were apparently very slight, and no attempt was made to determine them.

* In summarizing, the values of $(a)_D$ are given to the nearest 0.5°.

Effect of Solvent.—The following results obtained with the globulin from hemp-seed show that the salt solution used as a solvent was not without influence on its rotary power:

Solvent.	Value of $(a)_D$.
10 per cent sodium-chloride solution . . .	$-41.5°$
10 per cent ammonium-sulphate solution . .	$-40.0°$
10 per cent sodium-sulphate } solution . Equals 5 per cent anhydrous salt }	$-38.5°$

Alkali-albumins. — The differences in the specific rotations of the globulins studied have already been noted. Their alkali-albumins also differ in their rotary powers in the same order, as the following table shows:

Proteid.	Per cent Proteid.	Per cent NaCl.	Per cent KOH.	Value $(a)_D$.
Hemp-seed globulin . . .	0.75	9.0	0.8	$-61.7°$*
Brazil-nut globulin . . .	0.65	6.9	2.55	$-58.0°$†
Flax-seed globulin . . .	0.85	9.0	0.8	$-54.5°$

Alkaline and Acid Solutions. — The globulin from hemp-seed was the only one whose rotary power was investigated in dilute alkaline and acid solutions. In general, as with other proteids, the effect of the alkali or acid was to increase the specific rotation from 50 to 100 per cent, this increase being greatest in the acid solutions. Except in the sodium-carbonate solution, the increase in the rotary power took place almost immediately, and heating the solution did not materially alter its value. When dissolved in 0.2 per cent hydrochloric acid there was at first a large increase and then a slight decrease in the rotary power of the proteid. The following are the results obtained with alkali and acid solutions:

* This was the value of $(a)_D$ after heating the solution. Heating increased it.

† Heating this solution did not materially affect the value of $(a)_D$.

Solvent.	Per cent Proteid.	$(a)_D.$
0.18 per cent potassium hydroxide .	1.5	−64.5° within an hour −63.5° after heating
0.20 per cent sodium carbonate . .	2.2	−48.5° within an hour −50.0° after 6 hours −54.0° after heating
0.26 per cent hydrochloric acid . . .	1.8	−85.5° within 2 hours −81.0° after 20 hours −82.0° after heating −83.5° after 34 days

It is worth noting that the specific rotations of the animal and vegetable globulins, so far as known, lie between −38° and −48°, or perhaps within still closer limits. Besides those studied by the writer, the specific rotation of serum globulin is −48°, that of fibrinogen lies between −45° and −50°, and we have good reason for believing that that of egg globulin is about −40°.

In conclusion, the writer desires to acknowledge his indebtedness to Prof. R. H. Chittenden and Dr. L. B. Mendel, of the Sheffield Scientific School, for assistance and suggestions in the preparation of the proteids and their solutions; the success of this investigation is due in a large measure to their able and hearty co-operation. And also to Prof. C. S. Hastings, of the same institution, in whose laboratory the polarimeter observations were all made.

THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF SOME EDIBLE AMERICAN FUNGI.*

By LAFAYETTE B. MENDEL.

THE collection and spreading of information regarding fungi has lately received considerable attention. The efforts in this direction have been confined for the most part to descriptions of the common species, their peculiarities of growth and distribution. Especial consideration has been devoted to the variations among the fungi as regards toxicity; but accurate statements regarding chemical composition and possible nutritive value are largely wanting. The following extract from a recent monograph will serve to illustrate the current opinions. In referring to the edible mushrooms, it states: "The general opinion is, that mushrooms constitute a very nutritious and sustaining diet. Chemical analysis and personal experience indicate this. The former has shown that in their dry matter they contain from 20 to 50 per cent of protein or nitrogenous material. They may, therefore, well be called a kind of vegetable meat, and be used as a substitute for animal food. Like other vegetables they are largely composed of water, which is from 80 to 90 per cent of the whole. . . . The presence of so much nitrogenous material induces rapid decay and loathsome decomposition in them. . . . A hearty meal on mushrooms alone would be about as reasonable as a dinner on nothing but beefsteak, and might be expected to be followed by similar ill consequences."†

In view of the increasing importance of fungi as articles of

* Reprinted from the American Journal of Physiology, vol. I.

† Peck C. H., Report of the New York State Botanist, 1895, p. 113. Cf. also Peck, Mushrooms and their uses, 1897, p. 4. For the source of the statements quoted, Professor Peck has referred the writer to the Atlas of Champignons, by Richon and Rosé, and to Les Champignons, by Cordier — both of which it has been impossible to consult.

diet, the writer has gladly followed the suggestion of Professor Chittenden to ascertain something more definite regarding the composition of edible mushrooms, with particular reference to their nutritive qualities.

Methods. — Specimens were obtained from various sources, and in some instances different samples of the same species were examined.* The common methods of studying the composition of agricultural products have been adopted, the directions given by the Association of Official Agricultural Chemists being closely followed in most instances.† The mushrooms were cut up finely and thoroughly mixed. Samples were taken for the determination of moisture, while the bulk of the material was dried on a water-bath and then ground up to a fine powder. Dried to constant weight at 105° C., this served as material for analysis.

Ash was determined in the usual way, the incineration being carried on with the lowest possible heat. The mushrooms employed were previously cleaned with considerable care, and thus an excess of inorganic impurity, such as sand, was avoided.

Ether extract was obtained by treating the material with anhydrous and alcohol-free ether in a Soxhlet extractor for sixteen hours, the extract being finally dried in vacuo to constant weight. Recently Bugdanow‡ has shown that this method is insufficient to remove the last traces of fats completely from some vegetable materials, even when they are finely divided. The error is not sufficiently large, however, to affect the general conclusions from the analyses. In order to examine the extract for cholesterin it was saponified in the usual way with alcoholic potash. Cholesterin (or closely allied substances) was detected by Salkowski's reaction ; but the method of separation employed obviously does not exclude the possi-

---

* Acknowledgment is gratefully made of specimens obtained through the courtesy of Mr. Hollis Webster, of Cambridge, and Captain McIlvaine, of Philadelphia. The material used has in every case been identified, or verified, by Dr. A. W. Evans, to whom our thanks are due.

† See Wiley, H. W.: Agricultural Analysis, 1897.

‡ Bugdanow: Archiv f. d. ges. Physiol., 1897, lxviii, p. 408.

bility of this substance existing in combination with fatty acids in the fungi.*

Crude fiber was determined according to Wiley's method,† in the residue left after extractions with ether.

Total nitrogen was found by the Kjeldahl method, duplicate determinations always showing a very close agreement. It is customary in agricultural analysis to express the results thus obtained, and multiplied by the factor 6.25, as "crude protein." The latter term is thus made to include albuminoids and extractive bodies as well as the proteids proper.‡ Not only do these individual groups possess quite variable significance as foods, but this investigation has further demonstrated that such calculations may lead to quite erroneous conclusions. In the mushrooms, at least, a considerable part of the nitrogen probably exists as non-proteid nitrogen, a portion even belonging to the so-called crude-fiber, or cellulose elements of the fungi.§ In a large number of our analyses an attempt has been made to separate the nitrogen of the extractive bodies (amide-nitrogen, etc.) by treating a portion of the material repeatedly with boiling 85 per cent alcohol, so long as anything could be removed. The nitrogen content of the alcoholic extract having been determined, and then calculated on the material used, is designated as *extractive nitrogen.*‖ The amount of *alcohol-soluble material* was ascertained at the same time, by filtering the undissolved extraction residues on weighed filters and drying at 105° C. to constant weight. The difference between the total nitrogen and extractive nitrogen is provisionally given as *protein nitrogen*, though, as stated above, there is at present no justification for expressing

---

* Cf. Hürthle, K., Ueber die Fettsäure-Cholesterin-Ester des Blutes. Zeitschr. für physiol. Chemie, 1896, xxi, p. 352.

† Wiley, Agricultural Analysis, 1897, iii, p. 304.

‡ Cf. Atwater, W. O., Foods: Nutritive Value and Cost. Farmers' Bulletin, No. 23, pp. 5, 6. U. S. Dept. of Agriculture, 1894.

§ Winterstein, Berichte der deutsch. botan. Gesellsch., xi, p. 441; also Zeitschr. für physiol. Chemie, 1894, xix, p. 521; 1895, xxi, p. 134; Gilson, La cellule, xi, 1er fascicule.

‖ Cf. Mörner, C. Th., Zeitschr. für physiol. Chemie, 1886, x, 506.

the results as pure protein. Indeed, as will be pointed out later, this so-called protein nitrogen, in the present instance, contains a large proportion of nitrogen in a form wholly unavailable for the nutrition of the body.

Soluble carbohydrates were determined in an approximate manner by extracting the dry substance repeatedly with hot water and then boiling the extract for ten hours with hydrochloric acid of 2 per cent resulting strength. The sugar was determined as dextrose in the neutralized fluid, by the Allihn gravimetric method.

Experimental data. — *Coprinus comatus* (Shaggy coprinus). The specimens were freshly gathered and had not yet turned "inky." They varied very widely in size, thirty-six mushrooms weighing 1485 grams, of which 980 grams belonged to the caps (pileus) and 505 grams to the stems. The average weight of a fresh specimen was thus:

Pileus	27	grams
Stem	14	"
Total weight	41	"

A specimen which had attained the average growth weighed:

Pileus	43	grams
Stem	25	"
Total weight	68	"

An analysis yielded the following results:

Water	92.19	per cent
Total solids	7.81	" "

The dry substance contained:

Total nitrogen	5.79	per cent
Extractive nitrogen	3.87	" "
Protein nitrogen	1.92	" "
Ether extract	3.3	" "
Crude fiber	7.3	" "
Ash	12.5	" "
Material soluble in 85 per cent alcohol . .	56.3	" "

Coprinus atramentarius (Inky coprinus). Two separate, freshly gathered lots of this species were examined. The one (*a*) contained six young small specimens weighing 5.5 grams, or 0.9 gram each; the other (*b*) contained eight mushrooms weighing 12 grams, or 1.5 grams each. An analysis gave:

	a.	b.
Water	92.31 per cent	94.42 per cent
Total solids	7.69 " "	5.58 " "

The dry substance contained :

	a.	b.
Total nitrogen . . .	4.68 per cent	4.77 per cent
Ether extract . . .	3.1 " "	5.7 " "
Crude fiber	9.3 " "	. . . " "
Ash	16.8 " "	20.1 " "

Morchella esculenta (Common morel). Two lots of this species were obtained from Stockbridge, Mass. (*a*) The specimens were of full size. Thirteen morels weighed 195 grams, or an average of 15 grams each. (*b*) Small, young morels. An analysis gave :

	a.	b.
Water	89.54 per cent	91.24 per cent
Total solids	10.46 " "	8.76 " "

The dry substance contained :

	a.	b.
Total nitrogen	4.66 per cent	5.36 per cent
Extractive nitrogen	1.17 " "	. . .
Protein nitrogen	3.49 " "	. . .
Ether extract	4.8 " "	7.5 " "
Crude fiber	8.7 " "	9.5 " "
Ash	10.4 " "	13.6 " "
Material soluble in 85 per cent alcohol .	29.3 " "	. . .

In the same species Pizzi[*] has found 0.575 per cent nitrogen, a figure in close agreement with the above results when calculated upon the fresh material, viz. (*a*) 0.48 per cent N ; (*b*) 0.47 per cent N.

Polyporus sulphureus (Sulphury polyporus). The specimens were obtained from Pennsylvania. An analysis gave:

Water	70.80 per cent
Total solids	29.20 " "

The dry substance contained :

Total nitrogen	3.29 per cent
Extractive nitrogen	1.06 " "
Protein nitrogen	2.23 " "
Ether extract	3.2 " "
Crude fiber	3.0 " "
Ash	7.3 " "
Material soluble in 85 per cent alcohol . . .	27.8 " "

[*] Pizzi: Botanischer Jahresbericht, 1889, p. 316.

Pleurotus ostreatus (Oyster mushroom). This mushroom is obtainable in large quantities, and though somewhat tough in texture, is universally classed with the edible species. Specimens gathered from a tree in New Haven contained:

Water	73.70 per cent
Total solids	26.30 " "

The dry substance contained:

Total nitrogen	2.40 per cent
Extractive nitrogen	1.27 " "
Protein nitrogen	1.13 " "
Ether extract	1.6 " "
Crude fiber	7.5 " "
Ash	6.1 " "
Material soluble in 85 per cent alcohol . . .	31.5 " "

Clitocybe multiceps Peck. The material was collected near Boston, in June, 1897. A portion of small, young specimens was analyzed separately. The results follow:

	Young Specimens.	Full-grown Specimens.
Water	89.61 per cent	93.49 per cent
Total solids	10.39 " "	6.51 " "

The dry substance of the full-grown specimens contained:

Total nitrogen	5.36 per cent
Extractive nitrogen	3.38 " "
Protein nitrogen	1.98 " "
Ether extract	6.0 " "
Crude fiber	9.6 " "
Ash	11.5 " "
Material soluble in 85 per cent alcohol . .	57.2 " "

A portion of the mushrooms was separated into stems and caps and each analyzed separately, with the following results:

	Stem.	Pileus.
Water	94.07 per cent	92.68 per cent
Total solids	5.93 " "	7.82 " "
Total nitrogen in dry substance	3.92 " "	5.84 " "
Ash in dry substance . . .	12.98 " "	10.82 " "

The relatively higher content of nitrogen in the pileus corresponds with the distribution of proteid as shown by histochemical examination. In Agaricus campestris, Boletus edulis, and Boletus scaber, C. Th. Mörner has found similar differences between the nitrogen content of caps and stems.[*]

* Mörner, C. Th.: Zeitschr. für physiol. Chemie, 1886, **x**, p. 510.

Hypholoma candolleanum.* The specimens were obtained from East Milton, Mass., in June, 1897. A few small, young specimens were also obtained from Brookline, Mass. Analyses follow:

	Full-grown Specimens.	Younger Specimens.
Water	88.97 per cent	91.97 per cent
Total solids	11.03 " "	8.03 " "

The dry substance contained:

Total nitrogen	4.28 per cent	4.44 " "
Extractive nitrogen	1.79 " "	. . .
Protein nitrogen	2.49 " "	. . .
Ether extract	2.50 " "	. . .
Crude fiber	12.10 " "	
Ash	13.90 " - "	19.9 " "
Material soluble in 85 per cent alcohol	44.40 " "	. . .

Agaricus campestris (Common mushroom). Two varieties of the common mushroom were collected in New Haven. Fifteen specimens of one variety weighed 42 grams, an average weight of 2.8 grams each. The analysis gave:

	a.	b.
Water	87.88 per cent	92.20 per cent
Total solids	12.12 " "	7.80 " "
Total nitrogen in dry substance	4.42 " "	4.92 " "
Ash in dry substance	11.66 " "	17.18 " "

Regarding the differences in nitrogen content of cap and stem, compare the remarks under Clitocybe multiceps.

Marasmius oreades (Fairy-ring mushroom). Twenty freshly gathered specimens (from New Haven) weighed 9 grams, an average weight of 0.45 gram each. The analysis gave:

Water	74.96 per cent
Total solids	25.04 " "
Total nitrogen of dry substance	5.97 " "
Ash of dry substance	7.23 " "

Cortinarius collinitus (Smeared cortinarius). Young specimens gathered in New Haven early in November, 1897. The analysis gave:

Water	91.13 per cent
Total solids	8.87 " "
Total nitrogen of dry substance	3.63 " "

Digestion Experiments. — In order to procure further data regarding the nutrient value of the mushrooms, artificial

---

* The specimens corresponded with those described under this name by Stevenson in his work on British Hymenomycetes. Mr. Hollis Webster has informed the writer that Professor Farlow is inclined to regard them as H. apendiculatum.

digestion experiments were carried out with seven species of the fungi. The procedure was modified after the Stutzer method. About 2.5 grams dry substance were treated in a flask with 100 c.c. of an artificial gastric juice, containing 0.1 gram of very active scale pepsin and having an acidity of 0.35 per cent HCl. The flasks were frequently shaken, and after remaining in a thermostat at 38° C. for twelve hours, the undissolved residue was filtered off, washed free from acid, and again treated in the flask for several hours at 38° C. with 100 c.c. amylolytically active fresh chloroform-water extract of dog's pancreas, a little chloroform being added to prevent putrefaction or fermentation. Sodium carbonate (0.25 gram) was then added, followed by 25 c.c. of a proteolytically active thymolized extract of dry pancreas powder (Kuhne's method).* At the end of seven hours the residue was again filtered on a weighed filter, washed thoroughly with hot water, and dried at 105° C. to constant weight. *Undigested residue* was thus determined, and the nitrogen content ascertained by the Kjeldahl method and expressed as *nitrogen in residue*. The results expressed in percentages of dry substance are tabulated below.

Species Digested.	Dissolved Substance.	Undigested Residue.	Nitrogen in Residue.	Total Nitrogen.	Total Nitrogen Soluble.	Total Nitrogen Insoluble.
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Coprinus comatus . . .	73.79	26.21	4.21	5.79	4.69	1.10
" atramentarius .	71.84	28.16	2.79	4.68	3.90	0.78
Clitocybe multiceps . .	62.43	37.57	1.96	5.36	4.63	0.73
Hypholoma candolleanum	68.02	31.98	3.63	4.28	3.12	1.16
Morchella esculenta . .	50.58	49.42	4.16	4.66	2.61	2.05
Pleurotus ostreatus . . .	40.57	59.43	1.39	2.40	1.58	0.82
Polyporus sulphureus . .	45.00	55.00	1.05	3.29	2.71	0.58

Discussion of the Analytical Data. *Nitrogen and Protein.* From the results obtained it is evident that the nitrogen (and proteid) content of the mushrooms (or at least those species examined) is considerably smaller than is ordinarily stated.

* See Chittenden and Cummins, Studies from the Laboratory of Physiological Chemistry, Yale University, i, p. 109.

Thus Pavy, quoting from Payen's analyses, announces that in the dried state "mushrooms contain 52 per cent, morels 44 per cent, white truffles 36 per cent, black truffles 31 per cent, nitrogenous matter."[*]   In a number of species we have determined not only the total nitrogen, but also the extractive (non-proteid) nitrogen as well as the nitrogen in the residue insoluble after artificial gastric and pancreatic digestion.   The "protein" nitrogen multiplied — after deduction of the nitrogen in the undigested residue — by the factor 6.25 will give an approximation to the amount of proteid material available through the digestive processes going on in the alimentary canal, and thus throw some light on the true nutritive value of the mushrooms.   It is here assumed that the nitrogenous bodies soluble in alcohol are likewise soluble in the digestive fluids ; as to the possible presence of alcohol soluble proteids like zein, gliadin, etc., definite information is wanting at present.

The first table following gives a summary of the nitrogen content of various species; in the second table the amount of available proteid has been calculated in the manner referred to.

I.

The Percentages are Calculated on the Dry Substance.	Total Nitrogen.	Extractive Nitrogen.	"Crude protein" Nitrogen.
	per cent.	per cent.	per cent.
Coprinus comatus	5.79	3.87	1.92
Pleurotus ostreatus	2.40	1.27	1.13
Morchella esculenta	4.66	1.17	3.49
Hypholoma candolleanum . . .	4.28	1.79	2.49
Clitocybe multiceps	5.36	3.38	1.98
Polyporus sulphureus	3.29	1.06	2.23
Agaricus campestris—*a*	4.42	. . . .	. . . .
" " *b*	4.92	. . . .	. . . .
Coprinus atramentarius—*a* . . .	4.68	. . . .	. . . .
" " *b* . . .	4.77	. . . .	. . . .
Morchella esculenta (young) . .	5.36	. . . .	. . . .
Marasmius oreades	5.97	. . . .	. . . .
Cortinarius collinitus	3.63	. . . .	. . . .
Hypholoma candolleanum (young)	4.44	. . . .	. . . .

[*] Pavy, Food and Dietetics, 1881, p. 187.

II.

The Percentages are Calculated on the Dry Substance.	Nitrogen Insoluble in 85% Alcohol.	Nitrogen in Residue from Digestion.	Nitrogen of Proteid Dissolved in Digestion.	Digestible Proteid (N × 6.25).
	per cent.	per cent.	per cent.	
Morchella esculenta	3.49	2.05	1.44	9.00
Hypholoma candolleanum .	2.49	1.16	1.33	8.31
Coprinus comatus	1.92	1.10	0.82	5.12
Clitocybe multiceps	1.98	0.73	1.25	7.81
Polyporus sulphureus . . .	2.23	0.58	1.65	10.31
Pleurotus ostreatus	1.13	0.82	0.31	1.94

In considering the relatively high nitrogen content of the residue resisting digestion, it is to be noted that this is not necessarily derived from unattacked proteids. Winterstein [*] and others have shown that the "cellulose" preparations obtained by the usual methods from various fungi contain a considerable percentage of nitrogen; thus a preparation from Boletus edulis contained 5.5 per cent N, and this substance, like similar preparations from Agaricus campestris, Morchella esculenta, and other forms, yields glycosamin, $C_6H_{11}O_5 . NH_2$, on decomposition with HCl. It is thus allied to the chitin found in the animal kingdom; further investigation in this direction is highly desirable.

It is of interest in this connection to compare the results obtained by C. Th. Mörner [†] in an investigation of thirteen species of fungi common in Sweden. Nitrogenous constituents alone were considered, total N and extractive N, as well as digestible and indigestible N being determined by methods analogous to those used in the present research. Mörner's results, summarized in the following table, show a close agreement, in general, with those already given for different American species.

---

[*] Winterstein, loc. cit.; also, Berichte der deutschen chemischen Gesellschaft, 1894, xxvii, p. 3113; xxviii, p. 167.

[†] Mörner, C. Th., Zeitschr. für physiol. Chemie, 1886, x, p. 503.

The Results are Expressed as Percentage of Dry Substance.	N soluble in Pancreatic Juice.	N Soluble in Gastric Juice.	Digestible Protein-N.	Indigestible Protein-N.	Protein-N.	Extractive-N.	Total N.
Agaricus procerus (cap) .	0.28	2.71	2.99	1.27	4.21	2.02	6.23
Agaricus campestris (cap) .	0.35	3.29	3.64	1.17	4.89	2.49	7.38
Agaricus campestris (stem)	0.10	2.78	2.88	1.09	4.04	1.98	6.02
Lactarius deliciosus . . .	0.21	1.20	1.41	1.05	2.51	0.60	3.11
Lactarius torminosus . .	0.17	0.79	0.96	1.00	1.94	0.58	2.52
Cantharellus cibarius . .	0.08	0.71	0.79	1.46	2.29	0.40	2.69
Boletus edulis (cap) . . .	0.16	1.94	2.10	0.65	2.73	1.14	3.87
Boletus edulis (stem) . .	0.14	1.62	1.76	0.67	2.35	0.95	3.30
Boletus scaber (cap) . . .	0.18	1.48	1.66	0.85	2.54	0.58	3.12
Boletus scaber (stem) . .	0.12	0.87	0.99	0.62	1.71	0.48	2.19
Boletus luteus	0.22	0.48	0.70	1.06	1.77	0.74	2.51
Polyporus ovinus	0.08	0.42	0.50	0.84	1.35	0.45	1.80
Hydnum imbricatum . .	0.08	0.77	0.85	0.76	1.59	0.96	2.55
Hydnum repandum . . .	0.15	1.08	1.23	1.55	2.78	0.74	3.52
Sparassis crispa	0.09	0.37	0.46	0.40	0.97	0.21	1.18
Morchella esculenta . . .	0.22	1.97	2.19	1.90	4.18	0.81	4.99
Lycoperdon Bovista . . .	. . .	3.13	3.13	2.70	5.79	2.40	8.19

Ether Extract. — The amount of ether extract varied from 1.6 to 7.5 per cent in different species, as shown in the following summary of results.

ETHER EXTRACT.

Species.	Percentage calculated on Dry Substance.	Cholesterin.
Morchella esculenta (young) .	7.5	Present
Clitocybe multiceps	6.0	Present
Morchella esculenta	4.8	Present
Coprinus comatus	3.3	Present
Polyporus sulphureus	3.2	Present
Coprinus atramentarius . . .	3.1	Present
Hypholoma candolleanum . .	2.5	Present
Pleurotus ostreatus	1.6	Present

Gérard * examined the extract from Lactarius vellereus and L. piperatus, and found oleic and stearic acids present both as glycerides and as free acids. Volatile fatty acids were also

* Gérard, Journal de pharmacie et de chimie, 1890, 5 Série, xxi, p. 408; ibid. 1891, xxiii, p. 7. References to the earlier literature will be found in the first of these papers.

obtained, together with cholesterin or a closely related body (ergosterin), and evidences of lecithin. In the present research both fats and free fatty acids were found, and cholesterin reactions were obtained in every instance, the quantitative relations apparently varying considerably in the different species.

Alcohol Extract. — The following summary shows the amount of material soluble in warm 85 per cent alcohol in a number of species.

ALCOHOL EXTRACT.

The Percentages are Calculated on the Dry Substance.	Percentage of Soluble Material.	Percentage of Nitrogen Dissolved.
Clitocybe multiceps . . .	57.2	3.38
Coprinus comatus . . .	56.3	8.87
Hypholoma candolleanum	44.4	1.79
Pleurotus ostreatus . . .	31.5	1.27
Morchella esculenta . .	29.3	1.17
Polyporus sulphureus . .	27.8	1.06

Inorganic Constituents. — The amount of ash varied somewhat, as shown in the table below. Among the bases present, K, Na, and sometimes Ca are to be found, the K being quite abundant. Iron was always present. Of acids, phosphoric and sulphuric predominated, chlorine being occasionally found.

ASH.

The Percentages are Calculated on the Dry Substance.	Per cent.
Coprinus atramentarius,— a	16.8
" " — b (young) . . .	20.1
" comatus	12.5
Hypholoma candolleanum — a	13.9
" " — b (young) . .	19.9
Morchella esculenta — a	10.4
" " — b (young)	13.6
Agaricus campestris — a	11.7
" " — b	17.2
Clitocybe multiceps	11.5
" " (stems)	18.0
" " (pileus)	10.8
Polyporus sulphureus	7.3
Marasmius oreades	7.2
Pleurotus ostreatus	6.1

Crude Fiber. — Under this name is included the residue resistant to boiling acids and alkalis, and scarcely to be considered as homogeneous in nature. The results of the analyses are tabulated below.

It has already been pointed out that the cellulose of the fungi contains nitrogen in many instances, and Winterstein [*] has shown that the latter is not due to proteids or nucleins mechanically included; the nitrogen probably belongs to the " cellulose " itself. All attempts to separate the nitrogenous constituent from the portion which yields sugar on hydrolysis have failed.

CRUDE FIBER.

The Percentages are Calculated on the Dry Substance.	Per cent.
Hypholoma candolleanum	12.1
Clitocybe multiceps	9.6
Coprinus atramentarius	9.3
Morchella esculenta (young)	9.5
Morchella esculenta	8.7
Pleurotus ostreatus	7.5
Coprinus comatus	7.3
Polyporus sulphureus	8.0

Soluble Carbohydrates. — A considerable portion of the solids of the mushrooms is made up of soluble carbohydrates, while starch is ordinarily not found. Trehalose, a carbohydrate of the formula $C_{12}H_{22}O_{11}$, and resembling maltose in some respects, has been isolated from a number of species; [†] and in an extensive series of investigations Bourquelot [‡] has described a number of carbohydrates including mannite.

In order to get some idea of the amount of soluble carbohydrates present a number of experiments were carried out in the manner described under the methods of analysis. Since trehalose, for example, cannot be quantitatively converted

[*] Winterstein, Berichte d. deutsch. chem. Gesellsch., xxviii, p. 167; Zeitschr. für physiol. Chemie, 1894, xxix, p. 521.

[†] Winterstein, Zeitschr. für physiol. Chemie, 1894, xix, p. 70. The references to earlier literature are given.

[‡] These investigations were published in a series of papers in the Comptes Rendus and other scientific journals.

into dextrose by hydrolysis with acids,* the results of analysis must be somewhat low. Nevertheless the data may be of comparative interest as indicating a high content of soluble carbohydrate.

DEXTROSE FROM HYDROLYSIS OF WATER–SOLUBLE CARBOHYDRATES.

The Percentages are Calculated on the Dry Substance.	Per cent.
Pleurotus ostreatus	18.6
Coprinus comatus	18.0
Morchella esculenta	15.3
Polyporus sulphureus	12.2

To what extent these soluble carbohydrates are available for absorption in their natural form or after digestion it is impossible at present to say. Such qualitative tests as were made showed them to be transformed to reducing sugars rather slowly by the action of saliva. The large undigested residues (26–59 per cent) found in artificial digestions likewise suggest that they are not completely transformed in the alimentary canal. Reference may here be made to the observations of Stone † in feeding experiments on animals. He found that the pentosans, which are so widely distributed in vegetable foods, are to a marked degree less digestible than the carbohydrates, with which they have usually been indiscriminately classed in analyses.

After the presentation of the preceding analytical data it will scarcely be necessary to draw any elaborate comparison between the fungi and other well-known vegetable substances considered as food-stuffs. It may be well to emphasize the deficiencies of the methods commonly followed in estimating the proteid content of vegetable foods, and to call attention to the erroneous inferences which are consequently drawn regarding the nutrient value of these products. Thus it is not unusual in the construction of dietetic tables to multiply

* Winterstein, 1894, loc. cit., xix, p. 77.
† Stone, American Chemical Journal, 1894, xiv, p. 13.

the weight of nitrogen obtained by 6.25 and to express the result as "crude proteids." * But even where the precaution has been taken to remove non-proteid nitrogenous bodies by extraction with alcohol, the application of the " proteid factor " (6.25) to the N. of the residue may be quite misleading; for our results have demonstrated that the amount of unavailable nitrogenous material — largely, if not entirely, non-proteid in nature — is frequently equivalent to over half of the non-extractive nitrogen present (cf. Table II, p. 154). When it is remembered that the various species of mushrooms examined contain from 75 to 90 per cent of water, the amount of proteid in them appears strikingly small, even when calculated on the total nitrogen in the fungi.† For example, Morchella esculenta, a species of average composition as regards total solids (10.5 per cent) and nitrogenous constituents (0.48 per cent N) could contain as a possible maximum only 3 per cent of proteid, corresponding in this respect with potatoes, peas, green corn, etc.; ‡ the vegetarian would thus be obliged to consume several kilos of the fresh morel to obtain the daily requisite of 100 grams of proteid. The expression " vegetable beefsteak " accordingly seems scarcely appropriate when applied to mushrooms in a strictly chemical sense. Moreover, the comparative poverty of many species in proteids is corroborated by the results of other investigations now in progress in this laboratory, the yield of isolated substance being quite small. The fungi thus form no exception to the ordinary classes of fresh vegetable foods ; indeed, they take a decidedly inferior rank in comparison with many.

The carbohydrate content of the fungi is relatively high; but until more is known regarding the nature and digestibility of the carbohydrate constituents of various vegetable foods, it will be useless to draw comparisons. As dietetic accessories the edible fungi may play an important part; but investigation has demonstrated that they cannot be ranked with the essential foods.

---

* Cf. Wiley, Agricultural Analysis, 1897, iii, p. 543.

† Cf. Mörner, C. Th., Zeitschr. für physiol. Chemie, 1886, x, p. 515.

‡ Cf. Atwater, W. O., Foods : Nutritive Value and Cost, loc. cit., p. 27.

PAPAIN-PROTEOLYSIS, WITH SOME OBSERVATIONS ON THE PHYSIOLOGICAL ACTION OF THE PRODUCTS FORMED.*

By R. H. CHITTENDEN, LAFAYETTE B. MENDEL, and
H. E. McDERMOTT.

WHEN papain, the proteolytic enzyme of the papaw plant, was first subjected to careful study by Wurtz and Bouchut,[†] it was compared in its mode of action to trypsin, not alone because it was active in a neutral medium, but especially because of the character of the resultant products. Thus, it was stated that by the vigorous action of papain upon blood-fibrin, complete peptonization resulted, with the formation of some leucin in addition. Naturally, at this time (1879) there was no differentiation of proteoses and peptones; hence all that the above statement implied was a conversion of the proteid into a soluble form, precipitable by alcohol and not coagulated by heat nor by acids, although the presence of leucin would certainly suggest the formation of true peptone. Later, Martin [‡] pointed out that the enzyme acts vigorously in the presence of sodium carbonate (0.25 per cent) and that as products of digestion there are formed in both neutral and alkaline solutions an intermediate globulin-like body, peptone, leucin, and tyrosin, the last two being formed in small quantity. Here, likewise, the word peptone must be interpreted as meaning simply soluble proteid, and not carrying the dis-

† Wurtz and Bouchut, Sur le ferment digestif du Carica papaya. Comptes rendus, 1879, lxxxix, p. 425. Wurtz, Sur la papaine. Contribution à l'histoire des ferments solubles. Ibid., 1880, xc, p. 1379; and xci, p. 787.

‡ Martin, S. H. C., Papain-digestion. Journal of Physiology, 1884, v, p. 213.

tinction which is now known to exist between the proteoses and true peptones. Still later, however, Martin * studied the action of papain on the several proteids occurring with the enzyme in papaw juice, and found that the globulin present there was converted by the enzyme into an albumose (β-phytalbumose), and that this substance was transformed into a peptone-like body, which in turn was converted into leucin and tyrosin. In this case the peptone-like body referred to was presumably a true peptone in the modern acceptance of the term. Working with a somewhat different preparation of papain, the writer † observed incidentally that in the digestion of blood-fibrin and coagulated egg-albumin, deutero-albumose and true peptone predominated among the soluble products formed: *i. e.*, peptone, non-precipitable by saturation with ammonium sulphate. More recently Osswald ‡ has also reported that papain, as studied by him, gave rise to the formation of peptone in neutral, alkaline, and acid fluids, but that digestion was most complete and rapid in a hydrochloric acid solution. With regard to the latter part of the statement, we are inclined to believe that with most proteids the solvent action of papain is greatest in the presence of sodium carbonate and bicarbonate, although a mixture containing a very little hydrochloric acid may be more active than a neutral solution of the enzyme. Much depends, however, upon the presence or absence of extraneous matters in the ferment-preparation and on the amount of proteid present by which the presence or absence of *free* acid is determined. This question, however, is foreign to our present subject.

If the solvent action of papain on proteids is really due to conversion of the proteids into soluble albumoses and peptone, then its action must be compared with that of a true digestive enzyme and the process itself accepted as a genuine digestive

* Martin, The Nature of Papain and its Action on Vegetable Proteid. Journal of Physiology, 1885, vi. p. 337.

† Chittenden, Papoid-digestion. Trans. Conn. Acad. Arts and Sciences, 1892, ix, p. 321.

‡ Osswald, Untersuchungen über das Papain (Reuss). Münchener med. Wochenschr., 1894, No. 34.

process. In this connection it will be remembered that the corresponding vegetable enzyme bromelin, the proteolytic ferment of pineapple juice, is a true peptone-forming enzyme.[*] In fact, it resembles trypsin very closely in its ability, under suitable conditions, to transform the proteid undergoing digestion into true peptone. It is, of course, hardly to be expected that these vegetable enzymes will prove to be identical in every respect with the corresponding enzymes of animal origin. Indeed, we already know that in the action of bromelin there are certain minor differences at least in the primary or side-products formed as compared with those resulting from gastric and pancreatic digestion. There has, however, been no reason for doubting the ability of papain to form true peptone, although it must be admitted that since exact methods of separating albumoses or proteoses from true peptones have come into use, no one, so far as we are aware, has isolated the pure peptone or determined the extent or rate of its formation in papain-digestion. On the contrary, within the last few years, the statement has come from several sources that papain has no power whatever to form peptone; that its solvent or digestive action on proteids is limited to the production of proteoses and that peptone is never formed. Thus, Gordon Sharp [†] states that on warming coagulated egg-albumin with one-tenth its weight of papain and a hundred volumes of water for eighteen hours, no peptone could be detected either by saturating the digestive mixture with ammonium sulphate and testing the filtrate with the biuret test, or by dialyzing the digestive mixture and testing the diffusate (after one hour!) with phosphotungstic acid and by the biuret test. Albumoses, however, were formed. In a second communication [‡] the same writer states that by the action of papain upon

[*] Chittenden, On the Proteolytic Action of Bromelin, the Ferment of Pineapple Juice. Journal of Physiology, 1894, xv, p. 249.

[†] Sharp, Papain-digestion: Complete Absence of Peptone. Pharm. J. Transact., liii, p. 633, Edinburgh; Abstract in Chemisches Centralblatt, 1894, i, p. 512.

[‡] Sharp, The Action of Papain upon Egg- and Serum-Albumin in Acid and Alkaline Solution. Pharm. J. Transact., liii, p. 757, Edinburgh; Abstract in Chemisches Centralblatt, 1894, i, p. 830.

egg-albumin and serum-albumin in neutral, acid, and alkaline solutions peptone is never formed. Further, the opinion is expressed that the formation of peptone by papain is, on biological grounds, not to be expected, since the function of the ferment in the plant consists merely in transforming proteids into soluble compounds adapted for circulation through the open vessels, whereas in pepsin-digestion, on the other hand, the products of proteolysis must be adapted for absorption by osmosis prior to their distribution and utilization in the body. Lastly, it may he mentioned that Dott [*] in a comparative study of papain and pepsin has likewise found that the former enzyme, unlike pepsin, is not able to form peptone from egg-albumin. If these statements are correct, then, obviously, papain is quite different in its mode of action from other proteolytic enzymes, and the fact, if such it is, should be clearly established. There would seem to be no great difficulty in arriving at a definite conclusion regarding the matter, and the following experiments have been undertaken with a view to throwing some light upon the question.

In a preliminary experiment, coagulated egg-albumin (from a dozen eggs) was mixed with 800 c. c. of 0. 2 per cent sodium carbonate solution, 1 gram of commercial papain added, and the mixture, contained in a closed flask with a little thymol, warmed at 40° C. for three days. Further ferment action was then stopped by boiling, the undissolved matter removed by filtration, the filtrate neutralized with acetic acid, filtered from the precipitate which resulted, and further concentrated. From this concentrated fluid the proteoses were precipitated collectively and completely by saturating the fluid while boiling hot with ammonium sulphate, — carrying out the saturation in a neutral, acid, and ammoniacal fluid successively, as recommended by Kühne [†] for the complete separation of proteoses from peptone. On testing this proteose-free

---

[*] Dott, Comparison of the Digestive Action of Papain and Pepsin. Pharm. J. Transact., liii, p. 758, Edinburgh ; Abstract in Chemisches Centralblatt, 1894, i, p. 831.

[†] Kühne: **Erfahrungen über Albumosen und Peptone, Zeitschrift für Biologie, 1892, xxix, p. 1.**

filtrate with the biuret test, giving due heed to the necessity of adding sufficient potassium hydroxide to decompose all of the ammonium salt present, an intense reaction for peptone was obtained.   Indeed, it was quite evident from the character of the reaction that a fairly large percentage of true peptone had been formed.

A similar experiment was tried with coagulated blood-fibrin, this form of proteid being warmed at 40° C. for two days with 1 gram of papain in 800 c. c. of 0.4 per cent sodium carbonate, a little thymol being present.   On removal of the proteoses with ammonium sulphate, as described above, a strong biuret reaction was obtained in the filtrate, thus showing the formation of true peptone.

Obviously, one possible danger in experiments of this order, where an alkaline fluid containing so much admixed proteid is warmed at 40° C. for two or three days, is bacterial contamination by which putrefaction may be incited.   In the two preceding experiments, thymol was made use of to obviate this danger, but in the next experiment chloroform and sodium fluoride were likewise employed, as follows:

1	2	3	4
60 grams fibrin * 500 c.c. 0.25% Na_2CO_3 5 c.c. chloroform 1 gram papain	60 grams fibrin 500 c.c. 0.25% Na_2CO_3 2.5 grams thymol 1 gram papain	60 grams fibrin 500 c.c. 0.25% Na_2CO_3 5.0 grams NaF 1 gram papain	60 grams fibrin 500 c.c. 0.25% Na_2CO_3 5.0 grams NaF { 1 gram papain { boiled 5 min.

These mixtures were placed in suitably stoppered flasks, shaken thoroughly to insure complete solution of the sodium fluoride, etc., and warmed at 40° C. for twenty hours, with frequent agitation.   At the end of the period the mixtures were boiled and filtered, the filtrates neutralized, concentrated, and the proteoses separated as already described by saturation with ammonium sulphate.   On testing the filtrates with the

* Coagulated blood-fibrin.

biuret test, Nos. 1, 2, and 3 gave a strong reaction for peptone, the reaction in No. 3 being apparently a little the strongest. No. 4, in which the papain was boiled prior to mixing it with the fibrin, gave a purely negative result, thus showing that the peptone reaction in the preceding mixtures could not have come from any admixture contained in the papain itself, nor in the proteid made use of, and that consequently the peptone found must have been formed in some manner during the experiment. Further, this same negative result affords evidence that the peptone detected was not formed by putrefaction; hence it must come from the proteolytic action of the enzyme, which is plainly not hindered by the presence of either chloroform, sodium fluoride, or thymol. Lastly, it should be mentioned that the striking brilliancy of the peptone reactions obtained in Nos. 1–3 precludes the possibility of any other conclusion than that a fairly large proportion of true peptone was formed.

A similar series of experiments was carried out with coagulated egg-albumin, 75 grams of the moist coagulum being used in each mixture, with results wholly in accord with those just described. Further, another series in which fresh, thoroughly washed rabbit's muscle (60 grams in each mixture) was digested gave similar results, the only difference being that in Nos. 1–3 the peptone reaction was even stronger than with the coagulated proteids, as might perhaps be expected owing to the easier digestibility of the former. It is thus quite apparent that papain is a true peptone-forming enzyme, and furthermore is able to exert this action upon various kinds of proteid matter.

What now is the extent to which this formation of peptone may be carried by papain? In the digestion of proteids with pepsin-hydrochloric acid or gastric juice it has been clearly shown that the formation of peptone rarely exceeds 50 per cent; proteoses usually predominate.* With alkaline trypsin

---

* Chittenden and Amerman, A Comparison of Artificial and Natural Gastric Digestion, together with a Study of the Diffusibility of Proteoses and Peptone. Journal of Physiology, 1893, xiv, p. 483.

solution or pancreatic juice, on the other hand, the formation of peptone is much greater, although the hemipeptone formed is eventually broken down by the continued action of the enzyme into amido-acids, etc., leaving only the antipeptone. If papain is a true peptone-forming enzyme, related more closely to trypsin than to pepsin, it follows that under favorable circumstances it might be expected to produce even more than 50 per cent of peptone. It is not to be understood by this statement that papain can be compared with trypsin in rapidity of action; but merely that of the proteid dissolved by papain, under suitable conditions, fully 50 per cent might not unreasonably be looked upon as convertible into true peptone by the continued action of the enzyme. The correctness of this view has been tested by several series of quantitative experiments in which the proportion of proteoses and peptones formed has been determined as accurately as existing methods will allow.

The first experiment of this nature may be described as follows: Coagulated egg-albumin, formed by pouring the whites of eggs into boiling water acidified with acetic acid, was washed thoroughly with water, pressed, and finely divided. The content of dry albumin was then determined in a sampled portion by drying at 110° C., and igniting the residue to obtain the amount of ash. By this method 10 grams of the moist coagulum were found to contain 1.9257 grams of dry proteid. Three digestive mixtures were then prepared, each containing 150 c.c. of 0.25 per cent sodium carbonate saturated with chloroform, 50 grams of the moist coagulated albumin and 0.75 gram of active papain. To obviate any error that might be introduced through the presence of albumose, etc., in the papain, a fourth mixture was prepared similar to the above, except that it contained no albumin. All four mixtures were placed in closely stoppered flasks and transferred to a warm chamber, where they were kept at 38–40° C. for varying lengths of time with occasional agitation. One was allowed to digest for 25 hours, the second was interrupted at the end of 51 hours, while the third mixture

and likewise the control were continued for 75 hours. Diges-
tion was stopped by heating the mixture to boiling. It will
be noticed in these experiments that the proportion of papain
employed was quite small, considering the low digestive
power of the enzyme.

The mixtures were analyzed as follows: The undissolved
residue, made up largely of an insoluble antialbumid-like sub-
stance, together with some unaltered proteid, was collected on
a weighed filter, washed thoroughly with water and lastly with
alcohol, then dried at 110° C. until of constant weight. The
filtrate and washings were then neutralized with dilute acid,
and the neutralization precipitate so obtained was collected on
a weighed filter, washed with water until free from salts, dried,
and weighed. To determine the albumoses, the neutral filtrate
and washings were concentrated to a small volume and then
precipitated while still hot by saturation with pure neutral
ammonium sulphate, giving heed to Kühne's latest modifica-
tions of the method.* The precipitate was filtered by the aid
of a hot-water funnel and washed free from peptone with a hot
saturated solution of ammonium sulphate.† The precipitate,
together with the adherent ammonium sulphate, was then
washed into a weighed capsule with hot water, the mixture
evaporated to dryness, and finally dried in an air-bath at 110°
C. until of constant weight. Obviously, the weight so obtained
was the combined weight of the albumoses and ammonium sul-
phate. To ascertain the value of the latter, the mixture was
treated with water containing a little hydrochloric acid, the
fluid made up to a definite volume, and in an aliquot portion of
the latter the sulphuric acid was determined in the usual
manner by precipitation with barium chloride. From the
weight of barium sulphate thus obtained the amount of am-
monium sulphate was calculated and deducted from the com-
bined weight of the albumoses and ammonium salt. The
amount of true peptone formed was obtained in this experi-
ment by deducting the combined weight of the antialbumid

---

* Loc. cit.

† Continued until the filtrate failed to show any biuret reaction.

and undigested residue, neutralization precipitate, and albumoses from the weight of coagulated proteid used, making the necessary corrections for proteoses, etc., in the papain.

The results from this experiment were as follows, expressed in grams: —

Period of Digestion at 40° C.	25 hours.	51 hours.	75 hours.
Undissolved residue	3.4555	3.1626	. . . *
Neutralization precipitate . .	0.1692	0.0920	0.0075
Albumoses	2.6167	2.3700	1.2767
Dry proteid used	6.2414 9.6275	5.6246 9.6275	. . . 9.6275
Peptone formed	3.3861	4.0029	. . .

Expressed in percentages calculated on the dry proteid used, these figures yield the following results: —

Period of Digestion at 40° C.	25 hours.	51 hours.	75 hours.
Undissolved residue	35.8	32.8	. . .
Neutralization precipitate . .	1.7	0.9	0.1
Albumoses	27.1	24.6	18.3
Peptone	35.4	41.7	. . .
	100.0	100.0	. . .

In considering these figures, emphasis is to be laid upon the fact that the large percentage of undissolved residue noted above is by no means composed mainly of unaltered proteid, but is made up to a considerable extent of a peculiar alteration product which seemingly resembles antialbumid, the formation of which must involve a certain amount of energy on the part of the enzyme. Further, it is to be noted that at the end of twenty-five hours' digestion, 62.5 per cent of the proteid is converted into albumoses and peptone, while of these soluble products 56.6 per cent is composed of true peptone, the remaining 43.4 per cent being made up mainly of deuteroalbu-

---

* Lost by an accident.

mose. Moreover, it is seen that as the digestion is continued the proportion of albumoses decreases, peptones being correspondingly increased. To be sure, the figures representing the proportions of peptone formed are obtained by difference, but we see no reason why the methods pursued are not capable of yielding results substantially correct. Moreover, on testing the three ammonium sulphate-saturated filtrates containing the peptone with the biuret test, the intensity of the reactions obtained corresponded exactly with the above data. In this connection it should be mentioned that even under most favorable conditions the formation of amido-acids or other crystalline decomposition products by papain is very slight.

A second series of experiments similar to the above next demand attention because they help make clear possibly why some observers have failed to find evidence of the formation of peptone by papain. Four distinct mixtures were prepared, each containing 150 c. c. of 0.25 per cent sodium carbonate saturated with chloroform, 50 grams of moist coagulated egg-albumin, and 0.5 gram of papain. The fourth mixture, however, differed from the other three in that the papain was boiled with a portion of the fluid prior to mixing it with the albumin. It thus served as a control to check any possible errors that might arise from the action of the alkali alone on the proteid, or from soluble matter contained in the papain. Of greater importance, however, is the fact that the proportion of papain employed in this series of experiments was considerably less than in the previous series: *i. e.*, 0.5 gram instead of 0.75 gram for every 50 grams of proteid. Furthermore, the papain was a different sample, obtained from a different source, and had been tested solely as to its ability to *dissolve* proteid matter.

The several mixtures were kept at 38–40° C. for varying periods of time, one being removed at the end of 25 hours, the second at the end of 48 hours, while the third and fourth were continued for 72 hours. The mixtures were then analyzed as in the preceding case, with the following results expressed in grams: —

Period of digestion at 40° C. . .	25 hours.	48 hours.	72 hours.
Undissolved residue . . .	3.8354	3.9759	3.7577
Neutralization precipitate .	0.0405	0.0387	0.0100
Albumoses	2.8180	2.3278	2.5176
	6.6939	6.3424	6.2853
Dry proteid used	6.8564	6.8564	6.8564
Peptone formed	0.1625	0.5140	0.5711

In the control mixture, in which the papain had been boiled before mixing it with the albumin, 72 hours at 40°C. resulted simply in the formation of 0.03 gram of neutralization precipitate and a trace only of albumose. The undissolved residue when dried weighed 6.9301 grams, the plus weight being due to the insoluble matter of the papain. The slight corrections made necessary by these data have been embodied in the above figures.

The percentage results, calculated on the dry proteid used, are as follows: —

Period of digestion at 40° C.	25 hours.	48 hours.	72 hours.
Undissolved residue	55.9	57.9	54.8
Neutralization precipitation .	0.6	0.5	0.1
Albumoses	41.1	33.9	36.7
Peptone	2.4	7.7	8.4
	100.0	100.0	100.0

Here, for some reason, the formation of peptone was comparatively slight. Although the amount of papain employed in each mixture was less than the quantity used in the first series of experiments, the ratio of papain to dry proteid was much the same in the two cases. Evidently, the papain made use of in this last experiment was far less active than the preceding preparation, as shown also by the large percentage of undissolved residue. To be sure, considerable albumose was

formed, but the enzyme was so lacking in vigor that extensive proteolysis was impossible, and as a result the formation of peptone progressed very slowly. Still, even under these adverse conditions, some peptone was formed — easily recognizable by the biuret reaction — and the proportion increased slowly with continued digestion. There is therefore even in this experiment no confirmation of the statement that papain is unable to form peptone, but merely a suggestion of the necessity of obtaining an active preparation of the enzyme in order to arrive at a true understanding of its proteolytic power.

In a third series of experiments still another preparation of papain was employed : one which preliminary experimentation showed to be quite active. Each mixture contained 150 c.c. of 0.25 per cent sodium carbonate saturated with chloroform, 50 grams of moist coagulated egg-albumin, and 0.75 gram of papain. A control mixture of albumin, etc., in which the papain was boiled to destroy its activity, was also included in the series. In this series, however, digestion at 40° C. was continued for longer periods, and it is likewise to be noted that the ratio of dry proteid to the papain employed varied from that in the previous experiments. Further, the method of determining the albumoses and peptone was somewhat different from that previously used. Thus, after separating the undissolved residue and neutralization precipitate, the neutral fluid was concentrated and the albumoses precipitated by saturation of the fluid in the cold with pure zinc sulphate after the method of Bömer.[*] This precipitate was collected on a filter, washed thoroughly with a saturated solution of zinc sulphate, after which it was dissolved in water, the solution made up to a given volume, and the nitrogen determined in a fraction of the fluid by the Kjeldahl method. On multiplying the values so obtained by the factor 6.25 the amounts of albumoses present were calculated. The figures for peptone were obtained by difference, but were verified by determination of the nitrogen in the zinc sulphate-saturated filtrates.

---

[*] Bömer, Zinksulfat, ein Fällungsmittel für Albumosen, Zeitschr. f. analyt. Chem., 1895, p. 562.

This, however, was not easily accomplished owing to the presence of so much zinc sulphate; but on diluting the fluid with water we were able to determine the nitrogen in a small volume of the mixture, using the Kjeldahl method, and on multiplying the nitrogen found by the factor 6.25 we obtained values not greatly at variance with those given by difference. It is needless to say that the control mixture was treated in a similar manner and corrections made for nitrogen introduced with the papain, etc.   Following are the results obtained expressed in grams: —

Period of Digestion at 40° C.	48 hours.	98 hours.	144 hours.
Undissolved residue . . .	0.9487	1.0569	1.1136
Neutralization precipitate .	0.0542	0.0127	0.0152
Albumoses	0.6250	0.6665	0.7506
	1.6279	1.7361	1.8794
Dry proteid used	4.4042	4.4042	4.4042
Peptone formed	2.7763	2.6681	2.5248

Expressed in percentages, calculated on the dry proteid used, these figures lead to the following results: —

Period of Digestion at 40° C.	48 hours.	98 hours.	144 hours.
Undissolved residue	21.5	24.0	25.2
Neutralization precipitate . .	1.2	0.3	0.3
Albumoses	14.2	15.1	17.0
Peptone	63.1	60.6	57.5
	100.0	100.0	100.0

Here we have plain evidence again of the ability of papain under suitable conditions to form relatively large quantities of peptone, the latter, in this experiment, being greatly in excess over all the other products combined.   It is furthermore evident that in order to bring out the full proteolytic power of the enzyme (assuming an active preparation) it is necessary that the latter be present in fairly large proportion, *i. e.*, as compared with the proteid matter.   When this is the case, as

in the present experiment, the element of time is of less moment. In other words, when the ratio of enzyme to proteid is suitable, the maximum digestive action under those conditions is reached in 24–48 hours, and longer exposure at 40° C. fails to increase the proportion of peptone formed. In illustration of this point compare the results of the first and third experiments. In conclusion we think it clearly established that papain is not only a peptone-forming enzyme, but that under proper conditions it is able to transform a large proportion of the proteid matter into true peptone. In confirmation of this statement we have been able to prepare and isolate the pure peptone in quantity sufficient to study some of its physiological properties.

Some Observations on the Physiological Action of the Deuteroalbumose and Peptone formed by Papain.

It has been generally believed for some time past that the primary products which result from the proteolytic action of vegetable enzymes, as well as those formed by the action of superheated water, are somewhat different in nature from the corresponding products formed by pepsin-acid and by trypsin. Thus Neumeister * has shown that if atmid albumin or atmid albumose, *i. e.*, the albumose formed by the action of superheated water on blood-fibrin, is injected directly into the blood of a dog it appears in the urine wholly unaltered. An ordinary albumose, however, *i. e.*, such as is formed by pepsin or trypsin, when introduced into the circulation (of a dog) appears in the urine more or less hydrated.† Thus, protoalbumose appears in the urine in part as deuteroalbumose, while if deuteroalbumose is injected into the blood it appears in the urine as peptone. Peptone, on the other hand, is eliminated wholly unchanged. Neumeister ‡ also makes the statement

---

* Neumeister, Ueber die nächste Einwirkung gespannter Wasserdämpfe auf Proteine und über eine Gruppe eigenthümlicher Eiweisskörper und Albumosen. Zeitschr. f. Biol., 1890, xxvi, p. 77.

† Neumeister, Ueber die Einführung der Albumosen und Peptone in den Organismus. Ibid., 1888, xxiv, p. 272.

‡ Neumeister, Ibid., xxvi, p. 82.

that the products which result from the action of papayotin upon albuminous substances are identical with those formed by the action of superheated water. This implies that the so-called atmid products and the papayotin products are alike in their resistance to the action of pepsin,[*] for it is assumed at least that it is the presence of this enzyme in the kidney which leads to the hydration of the ordinary albumoses during their elimination from the body. In the case of rabbits, where pepsin is wanting in the kidney, the injection of albumoses into the blood is followed by their elimination unchanged (Neumeister).

Moreover, there are certain peculiarities in the chemical composition of the atmid bodies,[†] shared to some degree by the proteoses formed by the action of bromelin [‡] — the proteolytic enzyme of pineapple juice — which lends favor to the view that these bodies are not quite identical with the proteoses, etc., formed by animal enzymes. Consequently, it seemed desirable to study with some care the physiological behavior of the albumoses and peptone resulting from papain-digestion with a view to ascertaining what differences of a physiological nature, if any, exist between the latter products and those resulting from animal enzymes.

As has already been pointed out, the soluble products which are formed in the digestion of coagulated egg-albumin with papain are mainly deuteroalbumose and peptone. These were prepared in considerable quantity by digesting the coagulated albumin from four dozen hen's eggs with 9 grams of papain in 2 liters of 0.25 per cent sodium carbonate for 48 hours at

[*] Since this paper was written, there has appeared an article by E. Salkowski, "Ueber die Einwirkung des überhitzten Wassers auf Eiweiss," Zeitschr. f. Biol., 1897, xxxiv, p. 190 (Jubelband zu Ehren von W. Kühne), in which it is stated that the atmidalbumose formed by him from blood-fibrin was not resistant to the action of either pepsin, trypsin, or bacteria, thus differing widely from Neumeister's product.

[†] Chittenden and Meara, A Study of the Primary Products Resulting from the Action of Superheated Water on Coagulated Egg-albumin. Journal of Physiology, 1894, xv, p. 501.

[‡] Chittenden, The Proteolytic Action of Bromelin, the Ferment of Pineapple Juice. Ibid., 1894, xv, p. 249.

40° C. in the presence of chloroform. The resultant fluid freed from insoluble matter and neutralization precipitate was concentrated to a small volume and the albumoses precipitated by saturation with ammonium sulphate, boiling hot, from a neutral, acid, and alkaline reacting fluid. The precipitate so obtained was dissolved in water, the fluid carefully neutralized, and then dialyzed in running water until wholly free from ammonium sulphate and other salts. The solution was then filtered from a little insoluble matter (heteroalbumose, dysalbumose) concentrated to a small volume, and a portion tested for protoalbumose by saturation of the neutral fluid with rock salt. No precipitate whatever was obtained, consequently the entire volume of fluid was brought to a syrup and the deuteroalbumose precipitated with strong alcohol. After thorough washing with alcohol and ether, the substance was dried at 100° C. making about 20 grams of pure deuteroalbumose.

To obtain the peptone, the ammonium sulphate-saturated filtrate from the albumoses was treated with 50 per cent alcohol, thereby precipitating a large portion of the ammonium salt, while the residual sulphate was removed from the filtrate, after freeing from alcohol, by treatment with barium hydroxide followed by barium carbonate. On· evaporating the final filtrate to a syrup and treating with alcohol, the peptone was precipitated more or less gummy, after which it was dehydrated by successive treatments with absolute alcohol and ether, and finally dried at 100° C. About 10 grams of pure peptone were obtained.

Mode of Experimentation. — Our study of the physiological action of the deuteroalbumose and peptone formed above was limited to ascertaining their effects on blood-coagulation, their influence on blood-pressure, and their elimination by the kidneys. In all of the experiments dogs were employed, the animals always being anæsthetized. Most generally this was accomplished by means of a mixture of equal parts of chloroform and ether, although in some of the experiments morphine sulphate was injected hypodermically followed by the administration of chloroform and ether. In the few cases where

morphine was used, it was employed in the proportion of 1 centigram of morphine sulphate for each kilo of body-weight.

The albumose or peptone was introduced either into the left femoral vein or into the facial vein through a cannula connected with a burette. The substance, in the proportion of 0.5 gram per kilo of body-weight, was dissolved in 0.7 per cent sodium chloride solution, the volume of the fluid injected ranging from 30 c.c. to 50 c.c. and never exceeding the latter. The fluid was warmed to 40° C.

To observe the rate at which the blood coagulated, portions of about 5 c.c. each were withdrawn at stated intervals from the right femoral artery through a cannula inserted in that vessel, the blood being collected in slender test-tubes to observe the time of coagulation. Each time the blood was withdrawn from the artery the first portion passing out was discarded. Moreover, the cannula was removed and cleaned after each withdrawal of blood. Blood-pressure was registered at the carotid artery, or in some instances at the left femoral artery, using a Hürthle spring manometer and a Baltzar kymographion driven at a slow rate.

Influence on Coagulation of the Blood. — The effects of deuteroalbumose and peptone on the coagulation of the blood were observed in eight experiments on dogs ranging in weight from 5 to 11.5 kilos. In the first experiment the dosage of albumose was 0.33 gram per kilo of body-weight, but in four other experiments the dosage was increased to 0.5 gram per kilo, which proportion was likewise used in the three experiments with peptone. Following are the results obtained : —

<div align="center">

FIRST EXPERIMENT.

</div>

Dog, 9 kilos.   3 grams *deuteroalbumose* in 37 c.c. 0.7 per cent NaCl.

<div align="center">Injection lasted 2 min. 45 sec.</div>
<div align="center">The normal blood coagulated in 3 minutes.*</div>

Blood withdrawn 3 min. after injection of albumose coagulated in 30 min.

"	"	9	"	"	"	"	25 "
"	"	22	"	"	"	"	27 "
"	"	28	"	"	"	"	1–2 hours.

* The figure given for the coagulation-time of the normal blood is the average of 2–3 determinations.

SECOND EXPERIMENT.

Dog. 6.5 kilos.   3.25 grams *deuteroalbumose* in 50 c.c. 0.7 per cent NaCl.
Injection lasted 3 minutes.
The normal blood coagulated in 10 minutes.

Blood withdrawn 1 min. after injection of albumose coagulated in 1 hr. 27 min.

"	"	4	"	"	"	"	1	"	23	"
"	"	8	"	"	"	"	1	"	19	"
"	"	12	"	"	"	"	1	"	15	"
"	"	18	"	"	"	"	1	"	10	"
"	"	29	"	"	"	"	0	"	58	"
"	"	47	"	"	"	"	0	"	40	"
"	"	49	"	"	"	"	0	"	38	"

THIRD EXPERIMENT.

Bitch, 7.2 kilos.   3.5 grams *deuteroalbumose* in 50 c.c. 0.7 per cent NaCl.
Injection lasted 1 minute.
The normal blood coagulated in 9 minutes.

Blood withdrawn : —

2 min. after injection of albumose was uncoagulated at the end of 18 hrs.

8	"	"	"	"	"	"	"
25	"	"	"	"	"	"	"
46	"	"	"	"	"	"	"

FOURTH EXPERIMENT.

Dog, 7 kilos.   3.5 grams *deuteroalbumose* in 50 c.c. 0.7 per cent NaCl.
Injection lasted 1 min. 15 sec.
The normal blood coagulated in 3.5 minutes.

Blood withdrawn : —

6 min. after injection of albumose was uncoagulated at the end of 36 hrs.

12	"	"	"	"	"	"	"
18	"	"	"	"	"	"	"
24	"	"	"	"	"	"	"
34	"	"	"	"	coagulated within 5½ hrs.		
42	"	"	"	"	"	"	3 "

12

FIFTH EXPERIMENT.

Dog, 11.6 kilos.   5.6 grams *deuteroalbumose* in 40 c.c. 0.7 per cent NaCl.
Injection lasted 45 seconds.
The normal blood coagulated in 9 minutes.

Blood withdrawn 2 minutes after injection of albumose coagulated in 7½ hrs.

"	"	4	"	"	"	"	"		"
"	"	7	"	"	"	"	"		"
"	"	12	"	"	"	"	"	2 hrs.	36 min
"	"	17	"	"	"	"	"	2 "	31 "
"	"	26	"	"	"	"	"	0 "	42 "
"	"	36	"	"	"	"	"	0 "	20 "
"	"	45	"	"	"	"	"	0 "	23 "
"	"	55	"	"	"	"	"	0 "	13 "
"	"	65	"	"	"	"	"	0 "	6 "
"	"	75	"	"	"	"	"	0 "	8 "
"	"	85	"	"	"	"	"	0 "	2 "

SIXTH EXPERIMENT.

Bitch, 5 kilos.   3.7 grams *peptone* in 30 c.c. 0.7 per cent NaCl.
Injection lasted 40 seconds.
The normal blood coagulated in 3 minutes.

Blood withdrawn 5 minutes after injection of peptone coagulated in 6 hours.

"	"	9	"	"	"	"	"	"
"	"	15	"	"	"	"	"	"
"	"	24	"	"	"	"	"	1 hour.
"	"	55	"	"	"	"	"	45 min.
"	"	65	"	"	"	"	"	40 "
"	"	71	"	"	"	"	"	9 "

SEVENTH EXPERIMENT.

Bitch, 5.5 kilos.   2.75 grams *peptone* in 30 c.c. 0.7 per cent NaCl.
Injection lasted 30 seconds.
The normal blood coagulated in 1.5 minutes.

Blood withdrawn 3 min. after injection of peptone coagulated in 3–10 hours.

"	"	7	"	"	"	"	"	"
"	"	13	"	"	"	"	"	"
"	"	18	"	"	"	"	"	"
"	"	39	"	"	"	"	"	"
"	"	50	"	"	"	"	"	76 minutes.
"	"	60	"	"	"	"	"	50 "
"	"	80	"	"	"	"	"	30 "
"	"	90	"	"	"	"	"	5 "
"	"	98	"	"	"	"	"	7 "

EIGHTH EXPERIMENT.

Dog, 5 kilos. Control experiment. 30 c.c. 0.7 per cent NaCl.
Injection lasted 30 seconds.
The normal blood coagulated in 5 minutes.
Blood withdrawn 2 min. after injection of salt solution coagulated in 3 min.
" " 6 " " " " " 4 "
Fourteen minutes afterwards 3.0 grams *peptone* in 30 c.c. 0.7 per cent NaCl
were injected. Injection lasted 30 seconds.
Blood withdrawn 2 min. after injection of peptone coagulated in 10–17 hours.
" " 13 " " " " " "
" " 40 " " " " " "
" " 82 " " " " " " 3 hrs. 16 min.
" " 95 " " " " " " 0 " 10 "

From these experiments it is very manifest that both deuteroalbumose and peptone, as formed from egg-albumin by papain, have a marked effect upon the coagulation of the blood. With the dosage employed, namely, fifty centigrams per kilo of body-weight, coagulation is retarded for periods ranging from thirty minutes to thirty-six hours. Further, some of the experiments seemingly suggest that deuteroalbumose is somewhat more effective than pure peptone in retarding coagulation. It is likewise noticeable that this retarding effect upon coagulation is much more striking and also more permanent in some cases than in others, even though the conditions are apparently the same. Thus, in the second and third experiments, in which the dosage of deuteroalbumose per kilo is exactly the same, there is a marked difference in the character of the results, due, however, we believe, to differences in blood-pressure and to consequent differences in the rate of elimination through the kidneys. In connection with this last statement it is to be noted that in many of the experiments, with both albumose and peptone, the period of retardation shows a steady decrease (as in the fifth, seventh, and eighth experiments) until eventually, 50–100 minutes after the injection, the time of coagulation approaches somewhere near that of the normal blood.

What now is to be said regarding the relationship of these bodies in their action on blood-coagulation to the correspond-

ing bodies of animal origin? Obviously, in considering this question little weight can be attached to results obtained with such products as Witte's so-called peptone, since the latter, as is well known, is a mixture of several albumoses with some peptone. Hence, the earlier results obtained with products in which the two classes of substances — albumoses and peptones — were not differentiated have in the present connection only a general interest.* Pollitzer,† on the other hand, working with the individual albumoses formed by pepsin-acid, found that while all of these substances prevented or delayed the coagulation of the blood, the primary albumoses were most effective, deuteroalbumose least so. Further, amphopeptone led to variable results, frequently wholly negative, while antipeptone as formed by trypsin was almost entirely wanting in any constant effects. Grosjean,‡ however, observed that the peptone formed in gastric digestion does retard coagulation, although its action is less vigorous than that of the albumoses. With antipeptone, Spiro and Ellinger § found that the effect produced was dependent entirely upon the dosage of peptone employed. Thus, with 0.6 gram per kilo of body-weight coagulation-time was reduced from eight to four minutes, while with 1.1 grams of peptone per kilo the blood was rendered non-coagulable. Lastly, Thompson ‖ has

* Schmidt-Mülheim, Beiträge zur Kenntniss des Peptons und seiner physiologischen Bedeutung. Du Bois-Reymond's Archiv f. Physiol., 1880, p. 33; Fano, Das Verhalten des Peptons und Tryptons gegen Blut und Lymphe. Ibid., 1881, p. 277; Thompson, Contribution to the Physiological Effects of "Peptone" when Injected into the Circulation. Journal of Physiology, 1896, xx, p. 455.

† Pollitzer, On the Physiological Action of Peptones and Albumoses. Ibid., 1886, vii, p. 283.

‡ Grosjean, Recherches sur l'action physiologique de la propeptone et de la peptone. Archives de biologie, 1892, xii.

§ Spiro and Ellinger, Der Antagonismus gerinnungsbefördernder und gerinnungshemmender Stoffe im Blute und die sogenannte Peptonimmunität. Zeitschr. f. physiol. Chem., 1897, xxiii, p. 135.

‖ Thompson, The Physiological Effects of Peptone and its Precursors when introduced into the Circulation. Interim Report of a Committee consisting of Professors Schäfer, Sherrington, Boyce, and Thompson. Report by the Secretary, 1896–97.

reported that antipeptone in doses up to thirty centigrams per kilo tends to hasten the coagulation of the blood, while deuteroalbumose sometimes produces a retardation and sometimes a hastening of coagulation, apparently independent of the dosage.* It is obvious from these brief statements that any sharp comparison between the digestive products formed by papain and those resulting from the action of pepsin and trypsin is hardly possible. It is, however, seemingly true that the deuteroalbumose and peptone resulting from papain-digestion have a greater retarding effect upon blood-coagulation than the corresponding products formed by the animal enzymes. Thus, papain-peptone in doses of 0.5 gram per kilo never failed (in three experiments) to retard coagulation for 3–10 hours, while of antipeptone a dosage of 0.6 gram per kilo accelerates coagulation (Spiro and Ellinger). Further, papain-deuteroalbumose in doses of 0.33–0.5 gram per kilo invariably caused marked retardation of coagulation; far beyond anything reported by Thompson with deuteroalbumose in doses up to 0.3 gram per kilo. Any attempt at closer comparison in this direction would hardly be justified with our present knowledge. We would call special attention, however, to the tendency manifested in all of our experiments for the effect produced by papain-deuteroalbumose and peptone on the blood to pass gradually off, until finally, as in the fifth experiment, the coagulation-time may be considerably shorter than that of the normal blood. We attribute this result solely to the gradual elimination of the proteid, and as the rate of elimination varies with changes in blood-pressure, etc., produced by the substance, it follows that the duration of the effect upon the blood will vary not only with the dosage given, but also with the period of its detention within the blood current. Lastly, the acceleration of coagulation observed, 65–85 minutes after injection of the albumose (Experiment fifth) suggests that small doses of the substance may produce an effect quite the opposite of that produced by

* See also the papers on peptone and propeptone, by Gley and by Dastre in the Compt. rend. soc. de biologie, 1896.

a large dose, as observed by Spiro and Ellinger with antipeptone. In conclusion, we see in these results nothing to warrant the assumption that the two papain products are widely different from ordinary digestive products of a like degree of hydration. They certainly do not differ from the corresponding products of pepsin or trypsin digestion more than the latter products differ among themselves. Thus, according to the experiments of Arthus and Huber,* of gelatose, 2 grams per kilo of body-weight are required to render the blood of the dog non-coagulable, while of caseose 1.5 grams per kilo are needed; amounts far larger than are required of an albumose formed from either egg-albumin or blood-fibrin. Indeed, we have observed in a single experiment with pure protogelatose that 5 grams of the substance (dissolved in water) introduced into the facial vein of a dog weighing between three and four kilos, hastened the rate of coagulation.

Elimination by the Kidneys.—As already stated, Neumeister has shown that when ordinary albumoses are introduced into the blood of the dog, they are eliminated in the urine more or less hydrated. The atmidalbumoses, on the contrary, he found were eliminated unchanged. What now is the behavior of the deuteroalbumose formed by papain when similarly injected? In the second experiment already detailed, in which a dog of 6.5 kilos was given 3.25 grams of papain-deuteroalbumose by injection into the facial vein, the bladder (empty at the beginning of the experiment) was found one hour after the injection distended with urine. The fluid, amounting to 150 c.c., was removed, filtered, and saturated, boiling hot, with ammonium sulphate. A heavy gummy precipitate resulted, which after being washed with a saturated solution of the ammonium salt, was dissolved in water and tested. It was composed of unaltered deuteroalbumose. On testing the filtrate from the latter precipitate, after again boiling with ammonium sulphate to insure the complete removal of the deutero-

---

* Arthus and Huber, Action des injections intraveineuses de produits de digestions peptique et tryptique de la gélatine et du caséum sur la coagulation du sang chez le chien. Arch. de physiol., 1897, 5, viii, p. 857.

albumose, an intense biuret reaction was obtained, thus showing plainly the presence of a comparatively large amount of true peptone. In this experiment, therefore, there was marked diuresis, accompanied by a rapid elimination of the deuteroalbumose, but most important of all, a large proportion of the eliminated albumose underwent hydration into true peptone during its transit from the blood to the urine. Such a result as this, however, is not always obtained. Thus, in the third experiment blood-pressure was greatly lowered, and an hour after the injection the bladder contained only a few drops of fluid, with which no distinct reaction for either albumose or peptone could be obtained. In harmony with these two results, the blood in the last experiment drawn 46 minutes after the injection did not coagulate within 18 hours, while in the first experiment, where elimination was comparatively rapid, the blood drawn 49 minutes after the injection coagulated in 38 minutes. Further, in the fifth experiment detailed above, 85 minutes after injection of the albumose, retardation of blood coagulation was wholly at an end; indeed, coagulation took place more rapidly than prior to the injection. At this time the bladder was found distended with urine, and the latter gave a strong peptone reaction and a fair separation of albumose.* In the fourth experiment, there was no marked diuresis, but 76 minutes after the injection the bladder was half full of urine, the latter giving a strong reaction for peptone with only a trace of albumose. Thus, the results obtained in this connection certainly warrant the statement that whenever papain-deuteroalbumose undergoes elimination through the kidneys of the dog, it behaves in the same manner as an ordinary albumose, being transformed in great part into true peptone. It would seem, however, that injection of papain-deuteroalbumose is less liable to produce suspension of the renal secretion than injections of ordinary propeptone.†

---

* In a recent preliminary communication (Proceed. Physiol. Soc., Nov. 13, 1897), Thompson has likewise reported that Witte's "peptone" and Grosjean's peptone when injected into the jugular vein of dogs may lead to a marked increase in the quantity of urine accompanied by an excretion of part of the albumoses and peptone injected.

† Compare Grosjean, loc. cit.

With papain-peptone the elimination through the kidneys appeared less marked than with the albumose. Still, in all three experiments the bladder was found, 60–100 minutes after the injection, fairly well filled with urine, and on testing the latter a good biuret reaction for peptone was obtained. In no case was there any separation of an albumose precipitate on saturating the fluid with ammonium sulphate.

Influence on Blood-pressure.—Upon blood-pressure the albumose and peptone formed by papain from coagulated egg-albumin have much the same general effect as that produced by ordinary proteoses and peptone. In all of our experiments, in which the pressure was recorded, the dosage of albumose or peptone per kilo was somewhat larger than that ordinarily employed, namely, 0.5 gram. With this dosage, however, there was in nearly every experiment a marked and rapid fall of pressure lasting for about ten minutes. Moreover, the extent of the fall was seemingly influenced somewhat by the rapidity of the injection, a fact which has been commented upon by Thompson.[*] Our experiments also incline us to the belief that the character of the result may be modified somewhat by the personality of the animal, independent of the dosage and the duration of the injection. The experiments in this direction, however, were not intended to be exhaustive, but simply to throw light upon the main problem as to whether the papain products differ radically from ordinary digestive products. In one or two instances the fall of pressure was hardly noticeable, and in these cases the elimination of the albumose through the urine was quite rapid. The two following experiments may be taken as typical of what was generally observed with deuteroalbumose.

In a dog weighing 7 kilos, narcotized by chloroform-ether, 3.5 grams of deuteroalbumose dissolved in 50 c.c. 0.7 per cent NaCl solution were injected into the right facial vein, the injection lasting 1 min. 15 sec. The blood-pressure was lowered immediately from 160 mm. Hg to about 25 mm. Within four minutes, however, the pressure began to rise

* Thompson, Journal of Physiology, 1896, xx, p. 460.

gradually but steadily, and in ten minutes from the time of injection was approximately normal again.

A dog of 11.6 kilos, under chloroform-ether narcosis, was treated with 5.6 grams of the albumose by injection into the femoral vein, the injection lasting 45 seconds. Here, the pressure fell from about 150 mm. Hg to 100 mm. within one minute, gradually rising again to the normal in about seven minutes. In this experiment the fall of pressure was preceded by a slight rise, amounting to 5 mm. This initial effect of the injection of deuteroalbumose upon blood-pressure, i. e. a slight rise, is in harmony with the observations of Thompson* with Witte's " peptone."

Similar experiments with pure papain-peptone gave corresponding results, namely, an immediate and rapid fall of pressure, the latter rising to the normal again in nine to twelve minutes.

* Thompson, loc. cit., p. 461.

NOTES ON CETRARIA ISLANDICA (ICELAND MOSS).*

By ERNEST W. BROWN.

FROM early times lichens have been utilized as articles of diet for man and domestic animals.† First among them in importance as a food-stuff is " Iceland moss " (Cetraria islandica), which seems to have recommended itself because of its large content of carbohydrate matter, the so-called lichen-starch. In its natural form this lichen contains bitter constituents, and these must be removed by treatment with water or weak alkalies before the material can be made into bread, as has been the custom in some northern countries. Rabbits almost invariably refuse to eat the lichen unless it has been rendered more palatable as described.

With reference to the real dietetic value of Cetraria islandica, the following analysis of the commercial material will afford some data ‡:

ANALYSIS OF CETRARIA ISLANDICA (DRIED AT 105° C.).

Total nitrogen	0.56	per cent.
Extractive nitrogen	0.14	"
"Protein" nitrogen	0.32	"
Ether extract §	1.2	"
Crude fiber	5.3	"
Ash	2.2	"
Material soluble in 85 per cent alcohol	16.1	"
Soluble carbohydrates (as dextrose)	43.3	"

After successive treatment with gastric juice and amylolytically and proteolytically active pancreatic juice at 38° C. only 32 per cent of the material used was dissolved. The residue resisting digestion contained practically all the original nitrogen (0.55 per cent) of the lichen.

---

* Reprinted from the American Journal of Physiology, vol. i.

† Cf. Albert Schneider, A Text-book of General Lichenology, 1897.

‡ The methods of analysis employed were essentially the same as described by L. B. Mendel, Amer. Journ. Physiol., 1898, i, p. 226.

§ This consisted of free fatty acids (0.4 per cent) and saponifiable fat (0.62 per cent).

It will be observed that the quantity of proteids present must be small at most. The bulk of the material is made up of soluble carbohydrates. The latter were early made the subject of chemical investigation. Without attempting to recite the older and somewhat conflicting observations, we may refer to the more recent results of Hönig and St. Schubert.* These investigators conclude that extracts of Cetraria, obtained with hot water, contain two carbohydrates. The chief one of these, lichenin, forms a difficultly soluble jelly in cold water, an opalescent solution in hot water, is not colored blue by iodine, and does not rotate polarized light; on boiling with dilute acids lichenin yields crystallizable dextrose in addition to dextrins. The second carbohydrate, called lichenin starch, is regarded by these authors as a soluble modification of ordinary starch. It has also been called isolichenin.† Munk ‡ states that lichenin is most nearly related chemically to starch, and that it probably undergoes the same fermentative changes in the alimentary canal as are produced by boiling with dilute acids. The following experiments by the writer confirm in part and extend previous observations.

Lichenin. — *Preparation.* — The dry assorted Iceland moss was heated in a steam sterilizing apparatus for several hours with a considerable quantity of water, and the extract then filtered on hot water funnels. The cool filtrates deposited a thick jelly which was thrown upon filters and allowed to drain. The gelatinous mass was redissolved in hot water and reprecipitated repeatedly until the cold filtrates as well as the jelly no longer gave any blue coloration with iodine. The gelatinous substance was next treated with warm alcohol until all coloring matter was removed, then extracted with ether

* Hönig und St. Schubert, Sitzungsb. d. k. Akad. d. Wissenschaften zu Wien, 1887, xcvi, 2te Abth., p. 685. The older literature is referred to here. Cf. also Beilstein, Handbuch der organ. Chemie, 3te Auflage, i, p. 1098.

† Cf. Beilstein, loc. cit., p. 1099.

‡ Munk, J., und C. A. Ewald, Die Ernährung des gesunden und kranken Menschen, 1895, p. 102; also C. Voit, Die Ernährung. Hermann's Handbuch der Physiologie, 1881, vi, p. 413.

and dried. There resulted an almost white, tasteless, odorless powder, soluble in hot water, insoluble in cold water, free from nitrogenous matter, and yielding about $\frac{1}{2}$ per cent of ash.

Hydration by dilute acid. — In each trial a weighed quantity of lichenin was boiled for twelve hours with 2 per cent hydrochloric acid, and the resultant sugar determined in the neutralized fluid by the Allihn gravimetric method. The specific rotation was likewise ascertained and osazones were prepared.

I. 1.0936 grams lichenin (ash-free) yielded on hydration 1.097 grams dextrose. Assuming a hydration equivalent to that of starch, 1.0936 grams lichenin should yield 1.215 grams sugar.

II. (*a*) In a solution of hydration products containing 1.53 per cent sugar (determined as dextrose), in a 200 mm. tube an average of five polariscopic readings gave a rotation of +1.6°. Then $(a)_D = +52.2°$.

(*b*) In a solution containing 0.51 per cent sugar in a 220 mm. tube, an average of six readings gave a rotation of +0.6°. Then $(a)_D = +53.1°$. The specific rotation of dextrose, $(a)_D = +52.5°$.

III. The osazones of the sugar formed were prepared with phenylhydrazin in the usual manner, and recrystallized four times from alcohol. M. p. 199°–201° C.

The melting point of phenylglucosazone = 204° C.

The experiments thus indicate an almost complete hydration of lichenin, analogous in its results to the conversion of ordinary starch.

Action of enzymes and dilute HCl. —In order to determine the possible fate of ingested lichenin in the alimentary canal, the behavior of the carbohydrate towards the ordinary amylolytic enzymes was reinvestigated. The following typical experiments are selected from the protocols: —

I. A 1 per cent solution of lichenin in boiling water was prepared and placed in a bath at 38° C. Most of the material stays in solution; a portion separates out at this temperature. Saliva was added and the solution was tested for reducing sugars from time to time, with Fehling's solution. No reaction was obtained after *forty-five* minutes. To one portion ordinary starch paste (1 per cent) was now added. The solution reached the "achromic point" to iodine solution [*] in *one*

* Cf. Gamgee, Physiological Chemistry of the Animal Body, 1893, ii, p. 57.

minute and sugar was abundantly formed, thus showing that there was nothing present inhibitory to the action of the enzyme. The other portion of the original fluid was unchanged even after several hours.

II. A very active diastase preparation likewise failed to transform the lichenin to reducing sugar during an hour's action at 38°–40° C.

III. To a 1 per cent lichenin paste was added an amylolytically active pancreatic extract (alcoholic). No sugar was formed, while the unimpaired activity of the enzyme was demonstrated as in Experiment I.

IV. The ash from 1 gram of lichenin was added to a small quantity of starch paste. There was no inhibition of the subsequent action of saliva.

V. A 1 per cent lichenin paste was treated with saliva for an hour at 38° C. No sugar was formed. The solution was then precipitated with alcohol and the precipitate redissolved in water. The action of saliva was again tried, with the usual negative result. These operations were repeated four times with similar effects.

From experiments like the above it must be concluded that the ordinary amylolytic enzymes have no noticeable action on lichenin. Berg * is reported to have obtained similar results with saliva, malt diastase, pancreatic extract, and gastric juice. Since it has been shown that cane-sugar is readily inverted in the stomach by the gastric juice † and experiments in this laboratory have shown that inulin — likewise resistant to enzymes — is partly transformed to reducing sugar by the action of dilute HCl (0.2–0.4 per cent), the following experiment was tried : —

A 1 per cent lichenin paste was treated with an equal volume of 0.4 per cent HCl and kept at 38° C. for twelve hours. The test for sugar was negative. The mixture was carefully neutralized and treated with amylolytic pancreatic extract. No sugar was formed. Acid of 0.3, 0.4, and 0.5 per cent strength also gave negative results. Glycogen is likewise resistant to the action of these acids at 38° C.

Feeding experiments. — In view of the behavior of lichenin already recorded, it seemed desirable to ascertain whether this carbohydrate would give rise to a formation of glycogen in the liver, as has been found by Miura ‡ to occur after inulin feeding. Miura's experiments were followed as a type and protocols are given below—:

* Berg, Abstract, in Jahresbericht der Chemie, 1873, p. 848.
† Ferris and Lusk, Amer. Journ. Physiol., 1898, i, p. 277.
‡ Miura, K., Zeitschr. für Biologie, 1895, xxxii, p. 255.

Two rabbits, weighing 2.2 and 2.3 kilos respectively, were starved for six days. The control animal (2.3 kilos) was killed and the glycogen content of the liver found by the Brücke-Külz method to be 0.286 gram (0.7 per cent). The other rabbit (2.2 kilos) received 10 grams of lichenin, suspended in warm water, in five portions through the stomach sound at intervals of two hours. Twelve hours after the last portion was fed the animal was killed. The glycogen-content of the liver was found to be 0.086 gram (0.25 per cent). Another rabbit of 2 kilos, likewise starved, was fed about 8 grams of lichenin in several doses. The animal was accidentally killed immediately after a portion had been fed. The liver did not contain a weighable amount of glycogen.

The writer has not succeeded in finding rabbits that would eat any considerable quantity of the lichen itself, even after extraction with potassium carbonate to remove the bitter taste. Further experiments with larger quantities of lichenin are desirable.

Isolichenin. — This carbohydrate, to which is due the blue iodine-reaction in the filtrates from the lichenin preparation, has received little investigation.[*] It is in some respects closely related to soluble starch. The amount present in the lichen is decidedly less than the amount of lichenin, and a micro-chemical study shows it to be distributed through the cell walls of both the cortical and medullary portions of the plant. Micro-chemical reactions for cellulose give negative results.

Preparation. — The filtrates from the lichenin were concentrated in vacuo at a low temperature (35°–40°C.). If any remaining lichenin settled out on cooling it was filtered off and the solution was treated with several volumes of alcohol. The somewhat gummy precipitate was redissolved in hot water and again cooled. Further traces of lichenin were removed by filtration from the concentrated fluid; the isolichenin was reprecipitated with alcohol, extracted with alcohol and ether, and reduced to an almost white powder, containing 0.4 per cent ash. This preparation dissolves with difficulty in cold water, readily in hot water, from which it does not separate on cooling. With iodine solution it gives a blue coloration.

Hydration by dilute acid. — The following data were obtained by the methods already indicated for lichenin: —

[*] Cf. Berg, loc. cit.; Hönig und St. Schubert, loc. cit.

I. 1.021 grams isolichenin (ash-free) yielded on hydration 1.125 grams dextrose. Assuming a hydration equivalent to that of starch, the yield of dextrose should have been 1.134 grams.

II. (a) In a solution of hydration products containing 1.23 per cent sugar (determined as dextrose) in a 200 mm. tube, an average of six polariscopic readings gave a rotation of $+1.25°$. Then $(a)_D = +50.8°$.

 (b) In a solution containing 1.13 per cent sugar in a 200 mm. tube, an average of six polariscopic readings gave a rotation of $+1.17°$. Then $(a)_D = +51.7°$.

 The specific rotation of dextrose $(a)_D = +52.5°$.

III. The osazones of the sugar formed were prepared and recrystallized four times from alcohol. M. p. 199° C. The crystals resemble those of phenylglucosazone in appearance and solubility.

The hydration products of the isolichenin thus correspond closely in behavior with those obtained from the lichenin of the same plant.

Action of enzymes and dilute HCl. — Hönig and St. Schubert * subjected this carbohydrate to the action of malt diastase at 60° C. for several hours. They observed a rapid disappearance of the iodine reaction and formation of dextrin-like substance precipitable by alcohol. From such observations they class isolichenin — their lichen starch — with soluble starch. The writer has further studied the action of saliva, diastase, and pancreatic extract. Typical experiments are given below: —

I. A 1 per cent isolichenin solution was treated at 38° C. with saliva. The "achromic point" was reached in about one minute, no erythrodextrin stage being detected. Digestion was continued for an hour. The solution, tested from time to time, gave a slight reduction (with Fehling's solution) which did not increase in amount. Nylander's reagent gave no test for dextrose. The solution was precipitated with alcohol and the filtrate gave no reaction for sugars after removal of the alcohol. The precipitate of dextrin-like substance gave a slight reduction.† A flocky blue precipitate was always present in the test. Towards diastase and amylolytic pancreatic extract isolichenin showed similar behavior.

II. Isolichenin was treated with varying strengths of HCl (0.2–0.5 per cent) at 38° C. for twelve hours. No sugar was obtained in any instance.

* Hönig und St. Schubert, loc. cit., pp. 694–696.

† Musculus and v. Mering (Zeitschr. für physiol. Chemie, 1876, ii, pp. 410–419) obtained from glycogen and starch achroodextrins which likewise slightly reduce Fehling's solution.

The unusual behavior of isolichenin towards amylolytic enzymes — the formation of dextrins without sugars — recalls the formation (from glycogen) of dystropo-dextrin, an achroodextrin resisting the further action of enzymes.*

The peculiar carbohydrates of Cetraria islandica are doubtless merely types of those occurring in numerous other varieties of this group of plants.

* Seegen, Archiv f. d. ges. Physiol., 1879, xix, p. 106; Tebb, M. C., Journal of Physiology, 1898, xxii, p. 428.

THE INFLUENCE OF BILE AND BILE SALTS ON PANCREATIC PROTEOLYSIS.*

By R. H. CHITTENDEN and ALICE H. ALBRO.

THE natural commingling of bile and pancreatic juice in the duodenum is strongly suggestive of harmony of action, and it might reasonably be assumed that in pancreatic proteolysis the presence of bile would be in no wise inimical. Indeed, such few observations as have been recorded tend to show, as a rule, that the proteolytic action of the pancreatic enzyme is not materially impeded by the presence of bile or its constituent salts. Thus, many years ago Heidenhain † observed that when an aqueous solution of dried pig's bile was added to a glycerin extract of the pancreas, the proteolytic power of the latter was not diminished, but apparently increased. A similar stimulating effect was observed on addition of a 1 per cent solution of sodium glycocholate to the enzyme-containing solution. The few experiments then made were purely qualitative ones, proteolytic power being determined simply by noting the rate at which flocks of fibrin were dissolved. The results, however, were sufficiently convincing to lead Heidenhain to the conclusion that " the salts dissolved in the bile have an influence similar to that of sodium chloride." Some years later Lindberger ‡ found that the well known inhibitory action of organic acids upon trypsin proteolysis may be overcome, to some extent at least, by the presence of bile salts. Thus he observed that the presence of 1–2 per cent of bile with some sodium chloride would enable a trypsin solution containing 0.02 per cent of lactic acid to digest fibrin as rapidly as a neutral solution of the enzyme; indeed, as

rapidly as an alkaline solution, provided the content of alkali was not too great. If, however, the proportion of lactic acid was raised to 0.05 per cent, then bile and sodium chloride were without avail in stimulating proteolysis. Experiments made by the writer * some years ago likewise tended to show that the presence of bile in a pancreatic extract containing combined salicylic acid may increase somewhat the rate of trypsin proteolysis over that of the acid mixture alone. It was also shown at the same time that the addition of bile, even to the extent of 10 per cent, to neutral or alkaline pancreatic juice modifies only slightly the rate and extent of proteolysis; under some conditions inducing a slight stimulation and under other conditions a more marked inhibition of proteolysis. It was likewise observed that the deleterious action of combined hydrochloric acid upon trypsin proteolysis was not overcome by the addition of bile. A few experiments reported by Martin and Williams † have also tended to indicate that bile and bile salts may stimulate somewhat the rate of pancreatic proteolysis.

A careful survey of the results, and of the conditions under which the results were obtained, recorded up to this time, led the writer to the conclusion that the addition of bile to a neutral or alkaline pancreatic juice causes but little change in its proteolytic action. Some slight stimulation may be produced, but there is no convincing proof that this is of constant occurrence or sufficient in degree to possess much physiological significance. We have been more inclined to the view that while the presence of bile in the intestine may be of primary importance for the assimilation of fats, its action upon trypsin proteolysis is chiefly negative; *i. e.*, it neither retards nor stimulates proteolysis to any very great degree, under ordinary conditions. Recently, however, another paper ‡ bearing on this subject has appeared, which renders necessary a reconsideration

* Chittenden and Cummins, Influence of Bile, Bile Salts, and Bile Acids on Amylolytic and Proteolytic Action, Amer. Chem. Journal, 1885, vii, p. 50.
† Martin and Williams, Proceedings of the Royal Society, 1890, xlviii, p. 160.
‡ Rachford and Southgate, Medical Record, 1895, xlviii, p. 878.

of this question, for the results which the paper presents are so at variance with the above conclusions and so out of harmony with generally accepted views, that some explanation of the apparent divergence must be sought. This is all the more necessary from the fact that comparatively few systematic quantitative experiments have been tried. We have, therefore, attempted a thorough study of the subject with a view to establishing firmly the nature and extent of the action which bile and its constituents exercise upon pancreatic proteolysis.

In the experiments reported by Rachford and Southgate emphasis is laid upon the fact that they were " planned for the purpose of throwing some light on the proteolytic action of pancreatic juice, under the conditions which normally exist in the duodenum." With this end in view pure pancreatic juice was obtained from rabbits, through a pancreatic fistula, one rabbit yielding about 1 c.c. of the secretion in from four to six hours, this quantity sufficing for one experiment. As to the character of the bile employed there is no mention. We call attention to these facts because there is a manifest disposition on the part of these writers to accept the results of other workers in this field as conclusive for the pancreatic extracts, etc., employed, while their own divergent results are to be accepted as equally conclusive for the natural pancreatic juice. We are disinclined, however, to admit the correctness of this view. Pancreatic juice owes its proteolytic power to a specific enzyme. If the digestive power of this secretion is modified by the presence of bile through a specific action upon the enzyme, it is clear that this influence will be exerted whether we are dealing with the natural secretion or with an extract of the gland. If, however, the influence exerted is an indirect one, affecting the enzyme only through changes of reaction, etc., it is equally manifest that this influence can be detected and measured to the best advantage when the environment is thoroughly known. We see, therefore, no particular advantage in making use of the natural secretion in a study of this kind, especially where the volume available is so small as to render the attainment of accurate quantitative results somewhat

difficult. It is true theoretically that the addition of the fresh bile of an animal to the natural pancreatic juice of the same animal may constitute an ideal method for studying the influence of the former upon the activity of the latter; but when the exigencies of the case require that the digestive mixture be made by taking 5 drops of the natural pancreatic juice, adding 60 drops of water and 50 drops of a 4 per cent solution of bile,* we see very little reason for believing that the environment is thereby made to approximate any more closely to normal conditions than with the use of artificial extracts of the gland. The point involved is to our minds an important one, aside from the bearing it has upon the question before us. For if it is true that the action of a given agent upon a specific enzyme, or upon the specific power of the enzyme, is necessarily different, when added to the natural secretion in which the enzyme is contained from that which results when the same agent is added to an extract of the enzyme, then much of our knowledge regarding the conditions modifying and regulating the action of the digestive enzymes is of questionable value.

Rachford and Southgate seemingly incline to the view that the conditions under which their experiments were carried out approach closely those normally existent in the duodenum. Granting that this may be so, one is still inclined, after careful scrutiny of the conditions prevailing in their experiments, to wonder what the *actual* conditions really were. Nowhere is there any mention made of the reaction of the fluids employed, nor of the reaction of the resultant mixture when bile, hydrochloric acid, and pancreatic juice were combined. To state merely that a given digestive mixture was prepared by adding 8 drops of pure pancreatic juice, 30 drops of 0.1 per cent hydrochloric acid, and 50 drops of a 4 per cent solution of bile, leaves one in great doubt as to whether the mixture so manufactured was acid, alkaline, or neutral, and if acid whether it contained free or only combined acid. These are obviously very important elements to know if definite conclusions are to be drawn in explanation of the results. Data of this sort,

* Rachford and Southgate, loc. cit.

however, are wholly wanting in the paper in question, hence we are forced simply to take the results and guess at the actual conditions under which they were obtained. Further, so-called pure pancreatic juice, like the other digestive secretions, is subject to constant modification by the character and extent of the stimulation which calls it forth, while the character and condition of the semi-digested food passing from the stomach into the duodenum, together with the amount of free and combined acid, must necessarily lead to variable conditions in the duodenum. Add to this the well known variations in the rate of flow and composition of the bile, and we may well ask what are the conditions which normally prevail in the duodenum? Obviously, we cannot make a definite answer within very close limits, for the conditions are bound to be more or less variable. What we have to ascertain, therefore, is the influence of bile and its constituents upon the proteolytic action of the pancreatic juice or its specific enzyme under the various conditions which are liable to exist in the upper part of the small intestine. This, in our judgment, can be studied to the best advantage by the use of artificial pancreatic juice, or extracts of the active gland, where the quantity obtainable will be sufficient to admit of comparative experiments under definite conditions. Moreover, the artificial pancreatic juice will not be widely different in the character of the proteid matter present from the natural secretion employed by Rachford and Southgate. Thus, these investigators state that from four to six hours were required for about 1 c.c. of the pancreatic juice to flow from the fistula. Plainly, during this interval the active proteolytic enzyme present would transform the natural proteids of the juice into peptone, amido-acids, etc., as completely as the transformation would be accomplished in the extracts themselves. Hence, in this respect at least, both fluids must differ from the natural secretion normally poured into the intestine.

I. METHODS EMPLOYED.

The pancreatic extracts employed in our experiments were prepared from two kinds of glands and by two distinct

methods. 1. Using Kühne's well known method,[*] the fresh pancreatic glands of oxen were freed from fat and thoroughly dehydrated by long soaking in a large volume of strong alcohol and lastly in ether. To prepare the extract, 20 grams of the dry tissue were warmed at 40° C. for 24 hours with 200 c.c. of 0.1 per cent salicylic acid, the solution strained off, filtered through paper, made exactly neutral with sodium carbonate, and diluted with water to 1 litre. The sodium salicylate formed on neutralization will serve to prevent putrefaction for short periods, while the addition of a little thymol will preserve the fluid indefinitely. 2. Using Roberts's method,[†] pancreatic glands from pigs, freed from fat, were ground up with broken glass and soaked in four times their weight of 20 per cent alcohol for 4–5 days with frequent agitation. The extract was then filtered through paper, yielding a clear, slightly yellow fluid of strong proteolytic power.

The proteid material used in measuring the relative proteolytic power of the mixtures was purified blood fibrin, prepared by soaking carefully selected, well-washed fibrin in cold and boiling water until all soluble matter was removed, after which the fibrin was thoroughly extracted with cold and boiling alcohol and lastly with ether. The friable mass was then ground to a coarse powder and the latter passed through a series of sieves so as to bring together particles of the same size. It was then dried at 110° C. until of constant weight, and preserved for use. In some few experiments coagulated egg-albumin was made use of, in which case the actual content of dry proteid was determined by drying a given weight of the coagulum at 110° C. and then determining the ash by ignition.

The character of the bile employed is specified in each experiment. The secretion was always obtained as fresh as

---

[*] Kühne, Untersuchungen aus d. physiol. Institute, Heidelberg, 1878, I, p. 223.

[†] Roberts, On the Digestive Ferments, etc., The Lumleian Lectures, London, 1880.

possible, and when from dogs or cats it was usually obtained through a temporary biliary fistula.

In arranging the experiments each digestive mixture in a series was made to contain the same volume of pancreatic juice for each gram of dried fibrin — usually 10 c.c. or 20 c.c. according to the strength of the juice — with a total volume of 50 c.c.; water, bile, bile salts, acid, alkali, etc., being added in the proportions necessary to give the percentages specified. All the mixtures of a series were placed in the same water-bath at 40° C., all stirred equally, and when digestive action was sufficiently advanced the undigested residues were collected on weighed filters, previously dried at 110° C, in glass-stoppered weighing bottles, and washed thoroughly with hot water, and lastly with alcohol and ether. On drying the papers with their contents at 110° C. until of constant weight the proportion of undigested matter was readily ascertained, from which the relative proteolytic action of the several mixtures was easily calculated. It is hardly necessary to add that all the mixtures of a given series were kept at 40° C. for the same length of time, usually 2–4 hours, it being our aim to have the proteid material in the control mixture digested to the extent of 40–60 per cent.

II. Influence of Fresh Bile on the Proteolytic Action of the Pancreatic Enzyme in Neutral Solution.

The following quantitative results show the extent and character of the influence exerted by bile from various sources upon neutral solutions of trypsin: —

Experiment 1. Pig's bile. Neutral extract of pig's pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.4382 gram	56.18 per cent	100.0
0	0.4477	55.23	98.3
1.0	0.4891	51.09	90.9
3.0	0.4922	50.78	90.3
5.0	0.4616	53.84	95.8
10.0	0.4426	55.74	99.2
15.0	0.4204	57.96	103.1
20.0	0.4052	59.48	105.8
25.0	0.4206	57.94	103.1

Experiment 2.   Pig's bile.   Neutral extract of pig's pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.4218 gram	57.82 per cent	100.0
0	0.4330	56.70	98.0
1.0	0.4690	53.10	91.8
3.0	0.4705	52.95	91.5
5.0	0.4668	53.32	92.2
10.0	0.4528	54.72	94.6
15.0	0.4172	58.28	100.8

Experiment 3.   Pig's bile.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3823 gram	61.77 per cent	100.0
0.25	0.4039	59.61	96.5
0.50	0.4175	58.25	94.3
1.00	0.4948	50.52	81.7
2.50	0.4815	51.85	83.9
5.00	0.5100	49.00	79.3

Experiment 4.   Ox bile.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.2873 gram	71.27 per cent	100.0
1.0	0.3203	67.97	95.3
2.0	0.3203	67.97	95.3
3.0	0.3255	67.45	94.6
5.0	0.3306	66.94	93.9
10.0	0.3323	66.77	93.6
15.0	0.3019	69.81	97.9

Experiment 5.   Ox bile.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.6008 gram	39.92 per cent	100.0
1.0	0.5880	41.20	103.2
2.0	0.6049	39.51	98.9
3.0	0.6157	38.43	96.2
4.0	0.6287	37.13	93.0
5.0	0.6228	37.72	94.4
10.0	0.6179	38.21	95.7

Experiment 6.   Dog's bile.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.4070 gram	59.30 per cent	100.0
1.0	0.4165	58.35	98.3
2.0	0.4175	58.25	98.2
3.0	0.4348	56.52	95.3

Experiment 7.   Dog's bile.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3353 gram	66.47 per cent	100.0
0.25	0.3389	66.11	99.4
0.50	0.3295	67.05	100.8
1.00	0.3094	69.06	103.8
2.50	0.3579	64.21	96.6
5.00	0.3760	62.40	93.8

Experiment 8.   Dog's bile.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.4535 gram	54.65 per cent	100.0
0.25	0.4602	53.98	98.7
0.50	0.4541	54.59	99.8
1.00	0.4461	55.39	101.3
2.50	0.4370	56.30	103.0
5.00	0.4045	59.55	108.9
10.00	0.4188	58.12	106.3
15.00	0.4155	58.45	106.9

Experiment 9.   Cat's bile.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.4189 gram	58.11 per cent	100.0
0	0.4226	57.74	99.3
2.0	0.4453	55.47	95.4
7.0	0.4124	58.76	101.1

Experiment 10.   Sheep's bile.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.5161 gram	48.39 per cent	100.0
0.25	0.5098	49.02	101.3
0.50	0.5187	48.13	99.4
1.00	0.5271	47.29	97.7
2.50	0.5383	46.17	95.4
5.00	0.5018	49.82	102.9

Experiment 11.   Sheep's bile.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.2409 gram	75.91 per cent	100.0
0.25	0.2301	76.99	101.4
0.50	0.2709	72.91	96.0
1.00	0.2645	73.55	96.8
2.50	0.3011	69.89	92.0
5.00	0.3370	66.30	87.3
10.00	0.3142	68.58	90.3

Experiment 12. Sheep's bile. Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.4826 gram	51.74 per cent	100.0
0.25	0.4754	52.46	101.8
0.50	0.4717	52.83	102.1
1.00	0.4931	50.69	98.0
2.50	0.4685	53.15	102.7
5.00	0.4689	53.11	102.6
10.00	0.4383	56.17	108.5

Experiment 13. Sheep's bile. Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3425 gram	65.75 per cent	100.0
0.5	0.3356	66.44	101.4
1.0	0.3149	68.51	104.2
2.5	0.3258	67.42	102.5
5.0	0.3427	65.73	99.9
10.0	0.3287	67.13	102.0

Experiment 14. Sheep's bile. Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.5791 gram	42.09 per cent	100.0
0.25	0.5705	42.95	102.0
0.50	0.5637	43.63	103.6
1.00	0.5635	43.64	103.6
2.50	0.5772	42.28	100.4
10.00	0.5414	45.86	108.9

Experiment 15. Sheep's bile. Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3507 gram	64.93 per cent	100.0
1.0	0.3499	65.01	100.9
2.5	0.3492	65.08	101.0
5.0	0.3569	64.31	99.0
10.0	0.3132	68.68	105.7

Experiment 16. Sheep's bile. Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3305 gram	66.95 per cent	100.0
0.25	0.3282	67.18	100.3
0.50	0.3243	67.57	101.4
1.00	0.3344	66.56	99.4
2.50	0.3446	65.54	97.8
5.00	0.3398	66.02	98.6
10.00	0.3076	69.24	103.4

Experiment 17. Human bile.* Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3042 gram	69.58 per cent	100.0
1.0	0.2980	70.20	100.8
2.0	0.3175	68.25	98.0
3.0	0.3542	64.58	92.8
5.0	0.3652	63.48	91.2
10.0	0.3833	61.67	88.6
20.0	0.4335	56.65	81.4
40.0	0.4305	56.95	81.8

In looking through these various experiments it is manifest that the addition of fresh bile to a neutral pancreatic extract does not give rise to any very great degree of stimulation, *i. e.*, the proteid-digesting power of the enzyme is not markedly increased. Calling the digestive power of the control mixture without bile 100, it is rare to find the digestive power of the enzyme raised above 108 by addition of bile. Increased proteolysis, however, is certainly induced many times by the addition of bile, and it is somewhat noticeable that this increase is obtained more frequently in the presence of large percentages — 10–25 per cent — than in the presence of smaller amounts. Still, in no one of our experiments do we find a confirmation of the results reported by Rachford and Southgate, who found on an average " that the proteolytic action of pancreatic juice on neutral fibrin was increased one-fourth by the addition of bile." Further, it is noticeable in our experiments that in fully 50 per cent of the results inhibition of proteolysis is produced, although here, likewise, the retarding effect is not very marked. Rarely does the relative proteolytic action fall below 90. Our results, therefore, seemingly justify the statement that the addition of bile to a neutral solution of the pancreatic enzyme, even to the extent of 25 per cent, does not materially modify its proteolytic power; stimulation or inhibition may result, but not, under ordinary circumstances, to any very great degree. Still, the question arises at once why we have both stimulation and retardation. Obviously,

* Containing 17.96 per cent of solid matter.

some reason must exist for this apparent discrepancy in the results.   First, however, we must call attention to the extent to which our analytical data can be trusted.   Many times, to be sure, duplicate results agree very closely, but experience has taught us that, under the conditions of our experiments, the limit of error is about 1 per cent.   This means that where 50–60 per cent of proteid matter is digested, relative proteolytic action may vary two points without having any special significance (see Experiments 1, 2, 9, 18, 20, and 30).   It is plain, however, on carefully scrutinizing the preceding data, keeping in mind what has just been stated, that in some experiments bile manifestly tends to produce slight inhibition of proteolysis, while in other experiments, apparently under the same conditions, increased proteolysis results.   It is further manifest that this difference in action is to be connected mainly with the character of the bile employed.   Thus, it is to be noticed that increased proteolysis is much more common with sheep's and dog's bile than with ox or pig's bile.   Such differences in action might indeed be expected when it is remembered how radically bile from different species of animals differs in composition.   Still with sheep's bile stimulation of proteolysis is not constant, neither is inhibition always characteristic of pig's bile ; but this is not strange when we recall how the bile from a given animal may vary in composition with changes in physiological conditions.   Further, as Experiments 1 and 2 indicate, a given sample of bile may when present in one proportion retard proteolysis, while a larger percentage will accelerate digestion.   This suggests the possible presence in bile of two opposing factors, one tending to accelerate, the other tending to retard proteolysis.   Clearly such action as is produced cannot be due solely to the characteristic bile-salts which are contained in such abundance in the bile.

Bile is usually considered an alkaline-reacting fluid.   Thus, Neumeister * states that " the reaction of bile is alkaline; it contains about 0.2 per cent sodium carbonate and about the same amount of alkaline-reacting sodium phosphate."   It is

* Neumeister, Lehrbuch d. physiol. Chemie, 2te Auflage, 1897, p. 195.

true that fresh bile usually reacts alkaline to red litmus paper, but we have been unable to find any statements in the literature justifying the assumption that sodium carbonate is present. Indeed, some comparatively recent observations made by Jolles * show that the bile of oxen, dogs, and pigs, as well as human bile, reacts acid to phenolphthaleïn. This obviously does not imply the presence of free acid, although some free fatty acids may be present, such as stearic, palmitic, and oleic acids.† Jolles, indeed, concludes from his experiments that the fresh bile of man and the above-mentioned animals is not an alkaline or neutral fluid, but possesses a weak acid reaction. He finds, for example, on titrating ox-bile with a decinormal solution of potassium hydroxide, using phenolphthaleïn as an indicator, that on an average 1 gram of bile requires 0.546 milligram KOH to neutralize the free acids or acid-salts present. It is to be noted, likewise, that the acidity varies with different samples of bile, the extremes in ten experiments being 0.483 and 0.633. In pig's bile the average acidity was somewhat higher, 0.86 milligram KOH being required to neutralize the acid salts of 1 gram of bile. Further, the variations in acidity were much greater, the extremes in eight observations being 0.56 and 1.56. In dog's bile, on the other hand, the acidity (in one experiment) was only 0.42. Human bile, however, was much more strongly acid, 1 gram of bile requiring on an average 2.36 milligrams of KOH to neutralize the acid salts. These observations, which have an important bearing upon the subject under consideration, we are able to confirm in a general way through a large number of determinations made in this laboratory ‡ upon various kinds of bile. Only in rabbit's bile was there a failure to detect a measurable amount of acidity. Cat's bile, however, showed an acidity equal to only 0.23. Now, it is obvious from these statements that bile (excepting possibly rabbit's bile) cannot contain any alkali as strong as sodium

---

* Jolles, A., Archiv f. d. ges. Physiol., 1894, lxvii, p. 1.

† Lassar-Cohn, Zeitschr. f. physiol. Chemie, 1893, xvii, p. 607.

‡ By Ernest W. Brown.

carbonate. Such alkaline reaction as bile yields with red litmus, lacmoid, etc., must be due to the presence of such salts as Na_2HPO_4, NaH_2PO_4, etc. We may measure the amount of alkalinity in bile, using lacmoid as an indicator, by titration with a solution of decinormal hydrochloric acid. Using this method, Mr. Brown found on an average that pig's bile having an acidity equal to 0.50 milligram NaOH per gram had an alkalinity equal to 1.05 milligram HCl per gram. Sheep's bile with an average acidity of 0.45 possessed an alkalinity of 0.91. Ox bile with an average acidity of 0.43 showed an average alkalinity of 1.50, while rabbit's bile flowing directly from the liver through a fistula and not coming in contact with the gall bladder had an alkalinity of 2.9 without any measurable acidity. These statements would seem to show that the alkalinity as indicated by lacmoid is considerably greater than the acidity as indicated by phenolphthaleïn, but this is not always the case, for in some of our experiments, to be quoted shortly, it will be observed that the acidity frequently predominates. We would call special attention, however, to the observation made with rabbit's bile, for if it is true that the latter fluid invariably has a strong alkalinity, the addition of such a bile to neutral pancreatic juice would obviously accelerate proteolysis. If Rachford and Southgate used by chance the bile of the rabbit in their experiments, it might help explain the great acceleration of digestion noticed by them on addition of bile to "neutral fibrin." The point, however, which we wish to emphasize is that the bile of most animals under ordinary conditions is not a strongly alkaline fluid, that it contains no such alkali as sodium carbonate, but on the other hand is possessed of a weak acidity due to the presence of certain acid salts, such as the phosphates of the alkalies, together with possible weak organic acids or other organic compounds. The fact that the acid reacting bile fails to produce any effect on blue lacmoid is convincing proof that the fluid does not contain free organic acids of any strength. On the other hand, fresh bile does possess a certain degree of alkalinity to

be detected by litmus and lacmoid, but due mainly at least to the presence of salts or compounds which are either acid to phenolphthaleïn, or which exist side by side with such compounds. Lastly, we would emphasize the fact already brought out, that the so-called acidity of the bile, and likewise the so-called alkalinity, have different values in different species of animals, and may likewise vary in the same species under different conditions of diet, etc.

The bearing of these facts upon the problem before us is sufficiently manifest. The addition of bile to a neutral pancreatic fluid (neutral to litmus) must plainly introduce a change in the reaction, as measured by either litmus, lacmoid, or phenolphthaleïn. Further, owing to the variations in the acidity and alkalinity, already referred to, it is clear that different samples of bile will produce different results, and when it is remembered how sensitive the proteolytic enzyme trypsin is toward changes of reaction, it is obvious that this feature cannot be overlooked in considering the influence of bile upon pancreatic proteolysis.

Let us consider now the character of the bile used in some of the preceding experiments on proteolysis.

	Degree of Acidity.*	Degree of Alkalinity.†
Experiment 2. Pig's bile	0.50	1.17
Experiment 3. " "	0.60	. . .
Experiment 7. Dog's bile	0.90	0.54
Experiment 8. " "	0.76	0.40
Experiment 12. Sheep's bile . . .	0.50	0.37
Experiment 13. " "	0.40	0.91
Experiment 14. " "	0.60	0.73

These data show us at once that in introducing say 10 per cent of bile into the digestive mixtures, variations in reaction must necessarily ensue. Contrast, for example, the bile used

* Expressed in milligrams NaOH required to neutralize 1 gram bile, phenolphthaleïn as indicator.

† Expressed in milligrams HCl required to neutralize 1 gram bile, lacmoid as indicator.

in Experiments 2 and 7, also in 12 and 13.   Here we have marked differences in the ratio of acid salts to alkaline salts, but without any appreciable difference in the relative proteolytic action of the mixtures.   Everything else being equal, we should expect to find increased proteolysis most marked in those mixtures where the bile introduced showed a predominance of alkaline salts, but no such conclusion can be drawn from the results.   On the contrary, it is plain that such slight influence as bile exerts on the proteolytic action of *neutral* pancreatic juice is not connected primarily with change of reaction, but must be attributed to some other cause. These points, however, will be referred to again in another connection.

III. INFLUENCE OF FRESH BILE ON THE PROTEOLYTIC ACTION OF THE PANCREATIC ENZYME IN ALKALINE SOLUTION.

The experiments embraced under this head were conducted in the same manner as those previously described, except that to each digestive mixture was added 0.125 gram of sodium carbonate.   Hence, each mixture contained 0.25 per cent sodium carbonate, unless modified by the bile added.   Following are the results obtained: —

Experiment 18.   Pig's bile.   Alkaline extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3721 gram	62.79 per cent	100.0
0	0.3790	62.10	98.9
1.0	0.4440	55.60	88.5
1.0	0.4350	56.50	89.9
2.0	0.4490	55.10	87.7
2.0	0.4699	53.01	84.4
5.0	0.4318	56.82	90.4
5.0	0.4230	57.70	91.8
10.0	0.4255	57.45	91.4
10.0	0.4198	58.02	92.4

Experiment 19. Pig's bile. Alkaline extract of pig's pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3769 gram	62.31 per cent	100.0
1.0	0.3752	62.48	100.2
10.0	0.3997	60.03	96.8
15.0	0.4037	59.63	95.6
25.0	0.4349	56.51	90.6
50.0	0.5048	49.52	79.4

Experiment 20. Pig's bile. Alkaline extract of pig's pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.1535 gram	84.65 per cent	100.0
0	0.1498	85.02	100.4
3.0	0.2080	79.20	93.5
5.0	0.2177	78.23	92.4
10.0	0.2462	75.38	89.0
15.0	0.2400	76.00	89.7
20.0	0.2603	73.97	87.3
25.0	0.2428	75.72	89.4
30.0	0.2560	74.40	87.8
40.0	0.2743	72.57	85.7

Experiment 21. Pig's bile. Alkaline extract of ox pancreas.*

Per cent of Bile.	Undigested Residue.	Albumin Digested.	Relative Proteolytic Action.
0	0.0891 gram	88.41 per cent	100.0
0	0.0832	89.20	100.8
2.0	0.1320	82.80	93.6
5.0	0.0765	90.07	101.8
15.0	0.0257	96.66	109.3
20.0	0.0262	96.47	109.1

Experiment 22. Ox bile. Alkaline extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.2482 gram	75.18 per cent	100.0
1.0	0.2565	74.35	98.8
2.0	0.2771	72.29	96.1
3.0	0.2929	70.71	94.0
4.0	0.2860	71.40	94.9
5.0	0.2862	71.38	94.9
10.0	0.3036	69.64	92.6
15.0	0.2827	71.78	95.4

* In this experiment, thoroughly washed coagulated egg-albumin was employed instead of blood fibrin. Each mixture contained originally 3 grams of the coagulum = 0.7707 gram of dry proteid.

Experiment 23.   Calf's bile.   Alkaline extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.2996 gram	70.04 per cent	100.0
1.0	0.3190	68.10	.2
2.0	0.3409	65.91	94.1
3.0	0.3348	66.52	94.9
4.0	0.3314	66.86	95.4
5.0	0.3113	68.87	98.3
10.0	0.2662	73.38	104.6
15.0	0.2750	72.50	103.5

Experiment 24.   Human bile.*   Alkaline extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.2960 gram	70.40 per cent	100.0
0.5	0.3080	69.20	98.2
1.0	0.3091	69.09	98.1
2.0	0.3085	69.15	98.2
3.0	0.3074	69.26	98.3
5.0	0.3267	67.33	95.6
10.0	0.3360	66.40	94.3
20.0	0.3980	60.20	85.5
40.0	0.4904	50.96	72.3

Experiment 25.   Dog's bile.   Alkaline extract of pig's pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.1598 gram	84.02 per cent	100.0
0	0.1779	82.21	97.8
0.5	0.1855	81.45	96.9
1.0	0.2237	77.63	92.3
5.0	0.2041	79.59	94.7
10.0	0.2433	75.67	90.0
15.0	0.2481	75.19	89.4

Experiment 26.   Dog's bile.   Alkaline extract of pig's pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.1884 gram	81.16 per cent	100.0
2.0	0.1513	84.87	104.5
4.0	0.1148	88.52	109.0
8.0	0.1470	85.30	105.1
15.0	0.1804	81.06	99.8

* Containing 13.3 per cent of solid matter.

Experiment 27. Dog's bile. Alkaline extract of pig's pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3913 gram	60.87 per cent	100.0
5.0	0.3593	64.07	105.2
10.0	0.3622	63.78	104.6

Experiment 28. Dog's bile. Alkaline extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.5374 gram	46.26 per cent	100.0
20.0	0.5267	47.33	102.3

Experiment 29. Sheep's bile. Alkaline extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3935 gram	60.65 per cent	100.0
0.25	0.3880	61.20	100.9
0.50	0.4079	59.21	97.6
2.50	0.4236	57.64	95.3
5.00	0.4135	58.65	96.7
10.00	0.4002	59.98	98.8

Experiment 30. Sheep's bile. Alkaline extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.3284 gram	67.16 per cent	100.0
0	0.3330	66.70	99.3
0.25	0.3293	67.07	99.8
0.50	0.3328	66.72	99.3
1.00	0.3151	68.49	101.9
2.50	0.2937	70.63	105.1
5.00	0.2913	70.87	105.5
10.00	0.2635	73.65	109.6
15.00	0.2419	75.81	112.8

On looking through these results and comparing them with those of the preceding series, it is apparent that the addition of bile to an *alkaline* pancreatic juice is liable to produce a greater relative retardation of proteolysis than the addition of the same amount to a *neutral* pancreatic fluid. An alkaline pancreatic juice, however, containing even 40 per cent of bile will digest as much proteid in a given time as the same pancreatic fluid neutralized but without the addition of bile. If the control mixtures of the two series are compared, it will be observed that the alkaline fluids dissolve on an average about 10–15 per cent more proteid than the neutral fluids. This is

merely an illustration of the well-known fact that a weak alkaline fluid is much more favorable for pancreatic proteolysis than a neutral fluid. We are inclined to attribute such retardation of proteolysis as bile induces when added to an alkaline pancreatic fluid in great part to the reduction of alkalinity liable to occur. We say liable because this will depend primarily upon the relative proportion of acid and alkaline salts in the bile. When the latter contains a relatively large proportion of acid salts, as indicated by phenolphthalein, the addition of large percentages of such a bile will rapidly diminish the amount of sodium carbonate present, since the latter will be more or less used up in transforming the acid compounds into neutral ones. This is well illustrated in Experiment 20, wherein actual examination showed that at the conclusion of the digestion all the mixtures containing more than 15 per cent of bile had lost entirely their alkaline reaction toward litmus. In other words, the acid-reacting compounds contained in 10 grams of this bile was sufficient to neutralize the 0.125 gram of sodium carbonate originally present in the mixture. In harmony with this fact it is to be noted throughout these latter experiments that retardation is most marked in the presence of those biles which have been shown to have as a rule the highest acidity, viz., pig's bile and human bile. With dog's bile, on the other hand, in which acidity is usually very slight, no retardation whatever was observed; all three experiments gave evidence of some slight stimulation of proteolysis. The occasional stimulation noticed with sheep's bile and pig's bile we attribute in part to the lower acidity of the samples used. Leaving these points out of consideration, however, and turning our attention to the collected data, it is plain that the addition of even 40 or 50 per cent of bile to an alkaline pancreatic fluid does not greatly retard the proteolytic action of the enzyme (see Experiments 19, 21, and 24), certainly no more than would result from neutralization of the alkalinity. By this we do not mean that the specific bile salts are without influence on pancreatic proteolysis, but merely that the changes in reaction resulting from

addition of normal bile are in themselves sufficient to account for the retardation noticed. Similarly, such stimulation of proteolysis as results may be due as much to a more favorable change in the reaction of the mixture as to any other cause.

IV. Influence of Bile Salts on the Proteolytic Action of the Pancreatic Enzyme in Neutral and Alkaline Solution.

In considering the action of the salts of the bile acids on pancreatic proteolysis it is to be remembered that in ox bile both glycocholate and taurocholate of sodium are present, although the proportion of the two acids is subject to considerable variation. In most cases, when ox bile is acidified, the proportion of taurocholic acid present is sufficient to hold the less soluble glycocholic acid in solution, but in some cases the latter predominates to such an extent that it will crystallize out under the above conditions. Thus Marshall [*] found as the result of an examination of 543 samples of bile from as many oxen that a separation of glycocholic acid could be obtained in only 121 cases, *i. e.*, in 22.2 per cent. This fact is worthy of note in the present connection as illustrating not only the variable composition of bile from a given species, but also as an indication of the variability of composition to be expected in the preparation of " crystallized bile." [†] Further, in pig's bile we have to do mainly with the sodium salts of two special forms of glycocholic acid known as *a*- and *β*-hyo-glycocholic acid.[‡] In the preparation of crystallized bile, *i. e.*, the sodium salts of the above acids, the ordinary methods of procedure were followed, and need not be detailed here.

Following are the results obtained in studying the influence of these preparations on pancreatic proteolysis under the conditions specified : —

[*] Marshall, J., Zeitschr. f. physiol. Chemie, 1887, ii, p. 233.

[†] For the variation in the proportion of glycocholate and taurocholate present in human bile see the results reported by Hammarsten : Zur Kenntniss der Lebergalle des Menschen. Mitgetheilt der königl. Gesellsch. d. Wissenschaften zu Upsala, 15 Juni, 1893. Separatabzug.

[‡] Jolin, S., Zeitschr. f. physiol. Chemie, 1888, xii, p. 512, and xiii, p. 205.

Experiment 31. Crystallized ox-bile salts. Neutral extract of ox pancreas.

Per cent of Bile Salts.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.2933 gram	70.67 per cent	100.0
0	0.2707	72.93	103.1
1.0	0.2819	71.81	101.6
2.0	0.2579	74.21	105.0
3.0	0.3144	68.56	97.0
5.0	0.3790	62.10	87.8

Experiment 32. Crystallized ox-bile salts. Neutral extract of ox pancreas.

Per cent of Bile Salts.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.8866 gram	61.34 per cent	100.0
0.25	0.4481	55.19	89.9
1.00	0.4675	53.25	86.8
2.00	0.4651	53.49	87.2
3.00	0.4528	54.72	89.2
5.00	0.4360	56.40	91.9

Experiment 33. Crystallized ox-bile salts. Alkaline * extract of ox pancreas.

Per cent of Bile Salts.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.4940 gram	50.60 per cent	100.0
0	0.4970	50.30	99.4
1.0	0.4729	52.71	104.1
2.0	0.4465	55.35	109.3
3.0	0.4722	52.78	104.3

Experiment 34. Pure crystallized sodium glycocholate. Alkaline extract of ox pancreas.

Per cent of Glycocholate.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.5374 gram	46.20 per cent	100.0
0	0.5277	47.23	102.2
1.0	0.5459	45.41	98.3
2.0	0.5573	44.27	95.8
3.0	0.5987	40.13	86.8

Experiment 35. Bile salts from pig's bile. Neutral extract of pig's pancreas.

Per cent of Bile Salts.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.5781 gram	42.19 per cent	100.0
0.5	0.6197	38.03	90.1
1.0	0.6427	35.73	84.6
2.0	0.6894	31.06	73.6
3.0	0.7105	28.95	68.6

* Each mixture containing 0.25 per cent sodium carbonate.

Experiment 36. Bile salts from pig's bile. Alkaline extract of pig's pancreas.

Per cent of Bile Salts.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.4935 gram	50.65 per cent	100.0
0.5	0.4989	50.11	98.9
1.0	0.4750	52.50	103.6
2.0	0.4709	52.91	104.4
3.0	0.5383	46.17	91.1

The contrast between the results obtained in the two preceding experiments led to our testing the reaction of the bile salts prepared from pig's bile, and it was found that they were quite strongly acid to litmus. It is thus evident that the marked inhibition of proteolysis observed in Experiment 35 was due, in part at least to the increasing acidity of the mixtures. In Experiment 36, on the other hand, the 0.125 gram of sodium carbonate present overcame this acidity except in the mixture containing 3 per cent of the bile salts.

Experiment 37. Bile salts from pig's bile. Alkaline extract of pig's pancreas. These bile salts were exceedingly acid, hence 0.25 gram Na_2CO_3 was added to each mixture, thus making the amount of this salt 0.5 per cent in the control. In the presence of 3 per cent of the bile salts, however, the alkalinity was reduced to one-fifth.

Per cent of Bile Salts.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.1752 gram	82.48 per cent	100.0
0.5	0.2045	79.55	96.4
1.0	0.2142	78.58	95.2
2.0	0.2282	77.18	93.5
3.0	0.3281	67.19	81.4

Experiment 38. Bile salts from pig's bile, made neutral before addition. Alkaline extract of pig's pancreas. Each mixture containing 0.25 per cent Na_2CO_3, as usual.

Per cent of Bile Salts.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.1846 gram	81.54 per cent	100.0
0.5	0.2249	77.51	95.0
1.0	0.2207	77.93	95.5
2.0	0.2222	77.78	95.3
3.0	0.2324	76.76	94.1
5.0	0.2543	74.57	91.4

Experiment 39. Bile salts from pig's bile, made neutral before addition. Extract of ox pancreas.

Character of the Fluid.			Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
Neutral + 0		Bile salts	0.5499 gram	45.01 per cent	100.0
"	+ 1.0%	"	0.6467	35.83	78.4
"	+ 3.0%	"	0.6966	30.34	67.4
"	+ 5.0%	"	0.7701	22.99	51.0
0.25% Na_2CO_3 + 0		Bile salts	0.2748	72.52	161.1
"	" + 1.0%	"	0.3992	60.08	133.4
"	" + 3.0%	"	0.5238	47.62	105.7
"	" + 5.0%	"	0.6750	32.50	72.2

A critical examination of the foregoing results shows us first that the bile salts from ox bile have no very great influence in either direction upon pancreatic proteolysis. In two experiments (31 and 33) there is some evidence of acceleration, while in one experiment retardation is more noticeable. With pure sodium glycocholate (3 per cent) relative proteolytic action is reduced from 100 to 87. In Experiment 47, to be quoted later, stimulation of proteolysis is quite marked. In considering these results, however, in their bearing on the influence of fresh bile, it is to be remembered that 3–5 per cent of these bile salts are equivalent to the addition of 40–50 per cent of the original bile. With regard to the apparent difference in action of the several samples of ox bile salts we are inclined to attribute this to variations in the proportion of glycocholate and taurocholate present. Pure glycocholate, other influences•excluded, seems to have a greater inhibitory action than the mixed salts, and possibly the more pronounced retardation seemingly characteristic of the salts from pig's bile is due to the fact that the salts are mainly glycocholates. Still, it is to be observed that the samples of salts from pig's bile vary considerably in the intensity of their action, and this independently of their acidity, for when the latter is neutralized the same retarding effect is still produced. Somewhat noticeable also is the difference in intensity of action of the neutralized bile salts (from pig's bile) when added to the pancreatic juice of the same species as contrasted with

the result obtained when the salts are added to the pancreatic extract from another species (contrast Experiments 38 and 39). The results collectively certainly warrant the conclusion that the isolated bile salts taken by themselves do not exert any very marked stimulation of pancreatic proteolysis. They may, on the other hand, give rise to some retardation, — an effect which is seemingly more characteristic of the salts from pig's bile than of those common to ox bile. It is to be noted, however, that the salts from pig's bile were not so pure chemically as the crystallized salts separated from ox bile, but frequently showed an acid reaction.

The above somewhat unsatisfactory results have served to strengthen our conviction that such limited action as normal bile exerts on pancreatic proteolysis is the result mainly of influence on the reaction of the digestive mixture, and that many agencies other than the specific bile salts are concerned. No doubt, some of these are more or less antagonistic to each other. Thus, pig's bile, as has been frequently stated by many observers, is liable to be extremely viscid, but the viscidity is not always conspicuous ; at times the bile is quite limpid. This viscidity is due, in great part at least, to a mucin or nucleoalbumin, precipitable by alcohol, and we have found that when this substance is removed from the bile there is a noticeable difference in the influence of the fluid on proteolysis. We may cite the experiments on p. 218 : —

It is noticeable from these two experiments that the removal of the nucleoalbumin, with possibly some of the inorganic salts from pig's bile, diminishes in a general way the retarding effect of the latter on proteolysis. Somewhat noticeable also is the peculiar relationship in the rise and fall of proteolysis under the influence of different percentages of the two samples.

With ox bile an attempt was made to separate the fluid into three distinct fractions, using methods which would presumably cause little or no change in the nature or com-

Fresh pig's bile, very viscid, having a specific gravity of 1036, an acidity of 0.58, and an alkalinity of 1.15, was treated with five volumes of strong alcohol, the precipitate filtered off, and washed with alcohol. The united filtrate and washings were then evaporated for the removal of the alcohol, and the fluid made up with distilled water to the original volume. The acidity was now 0.48, and the alkalinity 0.90. The action of a portion of the original fresh bile and of the bile freed from nucleoalbumin, etc., on proteolysis was then tested.

Experiment 40.   With fresh pig's bile.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.5777 gram	42.23 per cent	100.0
0.25	0.6088	39.12	92.6
0.50	0.6241	37.59	89.0
1.00	0.7000	30.00	71.0
2.50	0.7118	28.82	68.2
5.00	0.6496	35.04	82.9
10.00	0.7235	27.65	65.4

Experiment 41.   With bile freed from nucleoalbumin.   Neutral extract of ox pancreas.

Per cent of Bile.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.6446 gram	35.54 per cent	100.0
0.25	0.6556	34.44	96.9
0.50	0.6651	33.49	94.2
1.00	0.7007	29.93	84.2
2.50	0.7351	26.49	74.5
5.00	0.7217	27.83	78.3
10.00	0.6598	34.02	95.7

position of the various constituents. For this purpose 440 c.c. of fresh ox bile, containing 12.4 per cent of solid matter, were evaporated to a very thick syrup on the water-bath and precipitated with absolute alcohol. The small precipitate which resulted was filtered off, washed thoroughly with alcohol, and then dried over sulphuric acid. It weighed 2.27 grams. The alcoholic filtrate was treated with a large volume of ether, the precipitated bile salts filtered off, washed thoroughly with ether, and dried. The alcohol-ether filtrate was allowed to evaporate, and finally brought to complete dryness on the water-bath. The effect of these three fractions on pancreatic proteolysis was then determined in the usual manner. The bile salts and the residue from the alcohol-ether

filtrate were readily soluble in water, but the alcoholic precipitate was not completely soluble. Following are the results obtained: —

Experiment 42. With a neutral extract of ox pancreas.

Per cent of Bile Constituents.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.4217 gram	57.83 per cent	100.0
0.50 Bile salts	0.4533	54.67	94.5
1.00 "	0.4452	55.48	95.9
1.50 "	0.4415	55.85	96.5
0.25 Alcoholic p.p. . . .	0.3862	61.88	106.1
0.50 " " . . .	0.3704	62.96	108.8
1.00 " " . . .	0.4059	59.41	102.7
0.50 Alcohol-ether filtrate	0.4147	58.53	101.2
1.00 " "	0.4032	59.68	103.2
1.50 " "	0.3759	62.41	107.9

Experiment 43. A duplicate of the preceding, except that an alkaline (0.25 per cent Na_2CO_3) extract of ox pancreas was employed.

Per cent of Bile Constituents.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
0	0.1886 gram	81.14 per cent	100.0
0.50 Bile salts	0.2847	71.53	88.1
1.00 "	0.2883	71.17	87.7
1.50 "	0.2655	73.45	90.5
0.25 Alcoholic p.p. . . .	0.2388	76.12	93.8
0.50 " " . . .	0.2345	76.55	94.3
1.00 " " . . .	0.2370	76.30	94.0
0.50 Alcohol-ether filtrate	0.2473	75.27	92.7
1.00 " "	0.2929	70.71	87.1
1.50 " "	0.2689	73.11	90.1

From the first of these two experiments, where a neutral pancreatic fluid was employed, it is seen that the fraction containing the bile salts produces a slight inhibition of proteolysis, while the other two fractions increase the proteolytic action of the enzyme. Also noticeable is the tendency of the material from the alcohol-ether filtrate to increase proteolysis in proportion to the amount added, while the favorable action of the alcoholic precipitate appears to diminish with increase in the proportion used. That these peculiarities of action, however, are not due to any direct influence upon the proteolytic enzyme is evident from the fact that in the second experiment where the reaction of the digestive mixtures is alkaline these

differences disappear.   The bile salts still produce inhibition, but the other two fractions no longer give rise to increased proteolysis; on the contrary, they tend to check the rate of proteolysis.   It is thus clearly evident that in bile there are present various elements capable under different conditions of producing divergent effects, in minor degree, upon pancreatic proteolysis — effects which may counterbalance each other to some extent.

V. Influence of Bile and Bile Salts on the Proteolytic Action of the Pancreatic Enzyme in the Presence of Free and Combined Acids.

It has been generally accepted, on the basis of what has seemed sufficient experimental evidence, that the proteolytic enzyme of the pancreas is practically inactive in the presence of free hydrochloric acid.*   Even *free organic* acids inhibit almost completely the action of the enzyme.   Further, the presence of combined acid, *i. e.*, combined with proteid matter, checks to a greater or less degree the activity of the digestive fluid.   Rachford and Southgate, however, state: " in our experiment we have found that the proteolytic action of pancreatic juice on fibrin is quite as strong in a $\frac{1}{30}$ per cent hydrochloric-acid solution as it is in a neutral solution.   If there is any difference, in fact, it is in favor of the hydrochloric-acid solution."   The statement is positive, but we cannot find in their paper any *conclusive* evidence as to the actual degree of acidity.   Thus, to quote one of their experiments, 10 minims of pure pancreatic juice, 50 minims of water, and 30 minims of 0.1 per cent hydrochloric acid were mixed together; — but how much of this acid was used in neutralizing the alkalinity of the pancreatic juice, and how much was combined with the proteids of the secretion?   Nowhere in their paper can we find any evidence of discrimination between free and combined acid, or any attempt to determine the actual

* Chittenden and Cummins, Studies in Physiol. Chemistry.   Yale Univer. 1885, vol. i, p. 135.   This paper contains full references to other work in this direction.

percentage of either free or combined acid, really present. The question is one of considerable physiological importance, and if it is true that the pancreatic juice acting in an acid solution will do more work than in a neutral solution, it should be clearly established. We have, therefore, first turned our attention to this point.

Experiment 44. With neutral extracts of ox pancreas and pig's pancreas (neutral to litmus).

10 c.c. of the extract of pig's pancreas required 4.95 c.c. 0.2 per cent HCl to combine with all the proteid matter present.*

10 c.c. of the extract of ox pancreas required 2.55 c.c 0.2 per cent HCl to combine with the proteids.

In the digestions with pig's pancreatic fluid each mixture contained 10 c.c. of the extract, while with the pancreatic fluid from the ox pancreas 30 c.c. of extract were used in each case. Acid was then added to the mixtures as specified, the percentages being calculated on the total volume (50 c.c.) of the digestive mixtures. In no case was any *free* acid present.

Ox Pancreatic Juice.			Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
Neutral			0.3279 gram	67.21 per cent	100.0
Proteids combined with HCl —					
Quarter saturated (0.007% HCl)			0.3782	62.18	92.5
Half	"	(0.015% HCl)	0.4995	50.05	74.4
Wholly	"	(0.030% HCl)	0.8710	12.90	19.1
Pig's Pancreatic Juice.					
Neutral			0.0435	95.65	100.0
Proteids combined with HCl —					
One-sixth saturated (0.0016% HCl)			0.0469	95.31	99.6
Quarter	"	(0.0025% HCl)	0.0550	94.50	98.7
Half	"	(0.0049% HCl)	0.0826	91.74	95.9
Wholly	"	(0.0099% HCl)	0.2239	77.61	81.1

Experiment 45. 10 c.c. of the extract of ox pancreas required 4.3 c.c. 0.2 per cent HCl to combine with the proteids.

10 c.c. of the extract of pig's pancreas required 4.6 c.c. 0.2 per cent HCl to combine with the proteids.

In the digestions, 10 c.c. of the extract of pig's pancreas and 30 c.c. of the extract of ox pancreas were employed.

---

* Tropæolin oo in methyl alcohol was used as the indicator for free acid. Obviously, in conducting the titrations, deduction was made for the excess of acid required to bring out the color.

Ox Pancreatic Juice.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
Neutral	0.3896 gram	61.04 per cent	100.0
Proteids combined with acid —			
One-eighth saturated (0.006 % HCl)	0.3951	60.49	99.0
One-sixth " (0.008 % HCl)	0.4798	52.02	85.2
One-fourth " (0.012 % HCl)	0.5030	49.70	81.4
One-half " (0.025 % HCl)	0.6509	34.91	57.1
Wholly " (0.050 % HCl)	0.9327	6.73	11.0

Pig's Pancreatic Juice.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
Neutral	0.1966 gram	80.34 per cent	100.0
Proteids combined with acid —			
One-sixth saturated (0.003 % HCl)	0.2110	78.90	98.2
One-fourth " (0.004 % HCl)	0.2654	73.46	91.4
One-half " (0.009 % HCl)	0.3281	67.19	83.6
Wholly " (0.018 % HCl)	0.5564	44.36	55.2

Experiment 46. With extract of ox pancreas. 25 c.c. of the neutral extract required 19.7 c.c. 0.4 per cent *salicylic acid* to combine with the proteids present.

Character of the Fluid.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
Neutral	0.4658 gram	53.42 per cent	100.0
"	0.4894	51.06	95.5
Proteids combined with acid —			
Completely saturated (0.157 % acid)	0.9860	1.40	2.6
" " (0.157 % acid)	0.9874	1.26	2.3
Half " (0.078 % acid)	0.8443	15.57	29.1
" " (0.078 % acid)	0.8375	16.25	30.4

In considering the results of the three preceding experiments it is to be remembered that in no one of the digestive mixtures was there any *free* acid present, hence such effects as are produced come solely from the influence of the combined acid. Of the latter, even a few thousandths of one per cent suffice to exert an inhibitory influence on proteolysis, and with a sufficient amount of combined acid alone, even with a weak organic acid, proteolysis may be almost completely checked. We fail to see, therefore, how the addition of acid to a *neutral* pancreatic juice can increase the digestive power of the solution.

What, now, is the influence of bile and bile salts on pancreatic proteolysis in the presence of combined acid? The answer to this question is found in the results of the following experiments: —

Experiment 47. With extract of ox pancreas. Ox-bile salts.

Character of the Fluid.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
	grm.	per cent.	
Neutral	0.5291	47.09	100.0
" + 2% Bile salts	0.5183	48.17	102.3
" + 4% "	0.4818	51.82	110.0
Proteids combined with acid —			
Eighth saturated with acid (0.005% HCl)	0.5860	41.40	87.9
" " " + 2% Bile salts	0.5589	44.11	93.6
Quarter " " (0.01% HCl)	0.6330	36.70	77.8
" " " + 2% Bile salts	0.6359	36.41	77.3
" " " + 4% "	0.6230	37.70	80.0

Experiment 48. With extract of pig's pancreas. Bile salts from pig's bile.

Character of the Fluid.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
	grm.	per cent.	
Neutral	0.1755	82.45	100.0
Proteids combined with HCl —			
Half saturated with acid (0.013% HCl)	0.2337	76.63	92.9
" " " + 0.5% Bile salts	0.4433	55.67	67.5
" " " + 1.0% "	0.4039	59.61	72.2
" " " + 2.0% "	0.4958	50.42	61.1
" " " + 3.0% "	0.4992	50.08	60.7

Experiment 49. With extract of pig's pancreas. Bile salts from pig's bile. The salts somewhat acid in reaction. Salicylic acid used to combine with the proteids.

Character of the Fluid.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
	grm.	per cent.	
Neutral	0.3041	69.59	100.0
" + 3% Bile salts	0.5338	46.62	66.9
Proteids combined with acid —			
Wholly saturated with acid (0.064% acid)	0.5795	42.05	60.4
" " " + 3% Bile salts	0.7608	23.92	34.3
Half " " (0.032% acid)	0.4296	57.04	81.9
" " " + 3% Bile salts	0.6773	32.27	46.3
Quarter " " (0.016% acid)	0.3608	63.92	91.8
" " " + 3% Bile salts	0.5591	44.09	63.3

Experiment 50. With extract of pig's pancreas. Bile salts from pig's bile made perfectly neutral. Salicylic acid used to combine with the proteids.

Character of the Fluid.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
	grm.	per cent.	
Neutral	0.2132	78.68	100.0
Proteids combined with acid —			
Half saturated with acid (0.053% acid)	0.6038	39.62	50.3
" " " + 0.5% Bile salts	0.7659	23.41	29.7
" " " + 1.0% "	0.8120	18.80	22.6
" " " + 2.0% "	0.8319	16.81	21.3
" " " + 3.0% "	0.8900	11.00	13.9

Experiment 51. With extract of ox pancreas. Fresh pig's bile. Salicylic acid used to combine with the proteids.

Character of the Fluid.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
	grm.	per cent.	
Neutral	0.4125	58.75	100.0
Proteids combined with acid —			
Half saturated with acid (0.038% acid)	0.7111	28.89	49.1
" " " + 0.5% Bile	0.7242	27.58	46.9
" " " + 1.0% "	0.7283	27.17	46.3
" " " + 5.0% "	0.7662	23.38	39.7
" " " + 10.0% "	0.7854	21.46	36.5
" " " + 20.0% "	0.7819	21.81	37.1

Experiment 52. Neutral extract of ox pancreas. Fresh ox bile.

In this experiment the proteids of the pancreatic extract were not treated with acid, but sufficient acid was added to the fibrin to saturate it, or half saturate it (as tested by tropæolin oo), prior to addition of the pancreatic extract.

Conditions.	Undigested Residue.	Fibrin Digested.	Relative Proteolytic Action.
	grm.	per cent.	
Neutral fibrin	0.3643	63.57	100.0
Fibrin saturated with acid (5 c.c. 0.2% HCl)	0.5755	42.45	66.8
" " " + 10% Bile	0.5490	45.10	70.9
" half saturated with acid (2.5 c.c. 0.2% HCl)	0.4690	53.10	83.5
" " " " + 5% Bile	0.4980	50.20	78.9

In only one of these experiments (Experiment 47) do we see any distinct suggestion of aid to pancreatic proteolysis when bile or bile salts are added to a pancreatic extract containing combined acid. Combined acid alone tends to retard proteolysis, and the addition of bile to such mixtures as a rule

increases still further the extent of retardation. Our results afford no confirmation whatever of the view that bile greatly aids pancreatic juice in its proteolytic action on acid fibrin. Neither are we inclined to believe "that pancreatic juice, plus bile, plus hydrochloric acid, can accomplish more work in proteolysis than can any other known pancreatic mixture." * If such were the case we fail to see why some evidence of such favorable action should not appear in our results. The inhibitory action of acids alone, and of acids and bile combined, on pancreatic proteolysis is not, in our judgment, to be looked upon as unfavorable to the normal digestive processes of the small intestine. What right have we to assume that the conditions existent in the normal duodenum are such as to require pancreatic proteolysis to take place in the presence of acid, either free or combined? The combined or free acid which passes from the stomach through the pylorus is without doubt quickly removed by absorption or destroyed by neutralization. The evidence is certainly in favor of the view that the contents of the duodenum are generally alkaline. This question has been admirably discussed in a recent paper by Moore and Rockwood,† in which also a large number of experimental data are offered, showing that in many animals at least, under different forms of diet, the contents of the intestine from pylorus to cæcum react alkaline. In some cases, to be sure, the contents closely adjacent to the pylorus were found to be acid, but when this was the case the acidity was usually limited to a few inches. Hence we are inclined to believe that pancreatic proteolysis as it occurs in the normal intestine takes place, to a great extent, in the presence of a neutral or alkaline reaction, and that under such conditions the proportion of bile ordinarily present is not inimical to the process.

* Rachford and Southgate, loc. cit.
† Moore and Rockwood, Journal of Physiology, 1897, xxi, p. 373.

ON THE EXCRETION OF KYNURENIC ACID.*

By LAFAYETTE B. MENDEL and HOLMES C. JACKSON.

ALTHOUGH it is nearly half a century since Liebig discovered kynurenic acid in the urine of the dog, and this compound has long been assigned the constitution of an oxyquinoline-carboxylic acid,† there is much investigation yet demanded regarding its antecedents and origin in the metabolic processes of the body. The occurrence of kynurenic acid in the animal organism is interesting, because with the exception of *a*-methylquinoline recently isolated by Aldrich and Jones ‡ from the anal secretion of Mephitis mephitica (common American skunk), it is, so far as we recall, the only quinoline compound discovered in connection with the animal body. Furthermore, the study of kynurenic acid production is important, because of the light which it promises to throw upon the transformations going on in the system, upon the constitution of the proteids from which the compound is derived, and possibly upon the physiological behavior of compounds like many of the alkaloids related to quinoline derivatives.

The early investigations on kynurenic acid can scarcely demand detailed consideration at present, since in the absence of satisfactory analytical methods the separation of uric acid (and possibly other substances) from the acid investigated was

† Schmiedeberg and Schultzen, Ann. Chem. Pharm., 1872, clxiv, p. 155. Kretschy, Berichte d. deutsch. chem. Gesell., 1879, xii, p. 1673; Monatshefte für Chemie, 1881, ii, p. 57.

‡ Aldrich and Jones, Journal of Experimental Medicine, 1897, ii, p. 439.

not accomplished.* It cannot be assumed that kynurenic acid completely replaces uric acid in the urine of the dog, inasmuch as the experiments of Solomin † have shown that both acids may occur together under appropriate conditions, and our own experience leads to a similar conclusion. Solomin found that although the uric acid nitrogen (determined by the Ludwig-Salkowski method) forms only a very small fraction of the total nitrogen excreted, the quantity of uric acid estimated per kilo of body weight may be as large as 0.01 gram, which corresponds with the average uric acid output per kilo in man. In the case of dogs in nitrogenous equilibrium, numerous experiments in this laboratory have given a considerably smaller excretion (0.003–0.004 gram per kilo).‡ The higher figures obtained by Solomin are perhaps attributable to the rather large quantities of proteid fed.§

Regarding the immediate origin of kynurenic acid, little of a positive character is to be found in physiological literature. Its close relation to the diet, and its ready production after the ingestion of meat, have frequently been pointed out. Thus Schmidt ‖ believed to have found kynurenic acid excretion to be greatest after feeding meat, and least with a bread diet, a milk diet yielding intermediate results. His figures for the various dietaries, are, however, by no means comparable, since the quantities of the typical food-stuffs ingested in the three periods were not at all equivalent.¶ The experiments of

* Cf. for example, Voit and Riederer, Zeitschrift für Biologie, 1865, i, p. 315.

† Solomin, Zeitschr. f. physiol. Chemie, 1897, xiii, p. 497.

‡ Cf. Chittenden, Journal of Physiology, 1891, xii, p. 220. Chittenden and Gies, Amer. Journ. Physiol., 1898, i, p. 1.

§ The daily diet of the 9-kilo dog consisted of meat, 400 grams; milk, 250 c.c.; and NaCl, 10 grams (loc. cit., p. 498). Regarding the increase in uric acid excretion following the ingestion of proteid, cf. Schultze, Arch. f. d. ges. Physiol., 1889, xlv, p. 401; Herter and Smith, New York Medical Journal, June 4, 1892; Hopkins, Schaefer's Physiology, 1898, i, p. 594.

‖ Schmidt, Ueber das Verhalten einiger Chinolinderivate im Thierkörper mit Rücksicht auf die Bildung von Kynurensäure. Inaugural-Dissertation. Königsberg, 1884. (Jaffé's laboratory.)

¶ The dog was fed (a) meat, 1 kilo, (b) milk, 2 liters, (c) bread, 1 pound, respectively, per day.

Schmidt and Rosenhain,[*] planned to observe the possible pro-
duction of kynurenic acid from various quinoline derivatives [†]
introduced directly into the organism, gave only negative re-
sults. The observations of Rosenhain and of Haagen,[‡] in
which a decrease of from 30 to 50 per cent of kynurenic acid
was obtained after administration of intestinal antiseptics
(*e. g.*, salol, thymol, naphthalin), suggested a connection be-
tween intestinal putrefaction and kynurenic acid excretion.
There is, however, no satisfactory evidence in these experi-
ments that kynurenic acid has its origin in the decomposition
going on in the intestine, since no data are given regarding
the direct action of the drugs administered upon the food util-
ization and body metabolism. Thus it seems quite possible,
in view of our experiments, that the diminished kynurenic acid
excretion observed after naphthalin administration, for example,
is to be attributed to poorer absorption of the proteid fed. It
may also be recalled in this connection how many drugs, *e. g.*,
antipyrin, antifebrin, salicylates, alcohol, exert a direct influ-
ence upon the production of uric acid ; and accordingly similar
specific effects may have been at work in Haagen's experi-
ments. Again Baumann [§] observed an undiminished excre-
tion of kynurenic acid in a dog in which several days' starving
and repeated doses of calomel had freed the intestine from
putrefactive processes as shown by the absence of ethereal
sulphates in the urine; while Haagen failed to find any de-
crease in kynurenic acid excretion after administering large
doses of iodoform, which exerts a pronounced action upon the
putrefactive processes in the intestine.[||] In this connection
we may point out that Nuttall and Thierfelder [¶] have lately

[*] Rosenhain, Beiträge zur Kenntniss der Kynurensäurebildung im Thier-
körper. Inaugural-Dissertation, Königsberg, 1886. (Jaffé's laboratory.) Cf.
also R. Cohn, Zeitschr. f. physiol. Chemie, 1895, **xx**, p. 210.

[†] E. g., carbostyril, quinaldin, oxymethyl-quinoline, kynurin, antipyrin.

[‡] Haagen, Ueber den Einfluss der Darmfäulniss auf die Entstehung der
Kynurensäure beim Hunde. Inaugural-Dissertation, Königsberg, 1887. (Jaffé's
laboratory.)

[§] Baumann, Zeitschr. f. physiol. Chemie, 1886, **x**, p. 131.

[||] Cf. Morax, Zeitschr. f. physiol. Chemie, 1886, **x**, p. 321.

[¶] Nuttall and Thierfelder, Zeitschr. f. physiol. Chemie, 1895, **xxi**, p. 109;
1896, **xxii**, p. 62.

demonstrated the possible origin of aromatic oxyacids in tissue metabolism, since they have found them in the urine of animals which were entirely free from all bacteria. It seems desirable to emphasize the preceding facts because the experiments of Haagen have repeatedly been misinterpreted and quoted in evidence of the intestinal origin of kynurenic acid,[*] although Haagen has carefully avoided such an interpretation of his observations.[†] Finally, the experiments of Capaldi [‡] have given additional evidence against the assumed intestinal origin of kynurenic acid.

In considering the immediate antecedents of kynurenic acid, tyrosin is at once suggested. The behavior of this aromatic compound with reference to its possible synthesis to oxyquinoline-carboxylic acid in the body has been investigated by Hauser [§] and Solomin,[‖] both of whom failed to obtain evidence of any direct relationship between the two substances.

Plan of present investigation. — The present investigation is an attempt to ascertain something more definite regarding the conditions which determine and modify kynurenic acid production and excretion. Unless otherwise stated, the data have been obtained with dogs. The animals were kept in suitable roomy cages which permit the separate collection of urine and fæces, and stand in a light, well-ventilated space. It was not found necessary to resort to catheterization, since the periods of observation always extended over more than one day and the animals soon became accustomed to discharge their urine

[*] Cf. for example, Hauser, Arch. f. exper. Pathol. u. Pharmakol., 1895, xxxvi, p. 3; also, Neumeister, Lehrbuch der physiol. Chemie, 2te Auflage, 1897, p. 721. " Eine ältere, von Baumann stammende Angabe, dass die Quantität der Kynurensäure von den Fäulnissprocessen im Darm unabhängig sei, scheint durch die neueren Untersuchungen widerlegt zu sein."

[†] Cf. Haagen, loc. cit., p. 26, " . . . so ist es zweifelhaft, ob die nach anderen Antisepticis, besonders nach Naphthalin, gefundene Verminderung der Kynurensäure auf Beschränkung der Darmfäulniss, oder ob sie nicht vielmehr auf anderen Umständen beruht."

[‡] Capaldi, Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 87.

[§] Hauser, Arch. f. exper. Pathol. u. Pharmakol., 1895, xxxvi, p. 1.

[‖] Solomin, Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 497.

with considerable regularity. The nitrogen was determined in the urine and diet by the Kjeldahl method; sugar, when present, was estimated by titration with Fehling's or Purdy's solution,* and kynurenic acid was found by the method of Capaldi,† which has proved very satisfactory. The product thus obtained always responded to Jaffé's test ‡ and was crystalline: in a few urines a very small quantity of an amorphous substance was precipitated, which failed to give the characteristic reaction. As an immediate test for kynurenic acid — in the urine — the bromine water reaction, first recommended by Baumann,§ was frequently found useful. Bromine, as is well known, usually gives an insoluble yellow precipitate when added to dog's urine, the composition of the precipitate depending upon the presence of phenol bodies, indol, or kynurenic acid. With a little experience it becomes easy to make use of the reaction in judging the relative amounts of kynurenic acid, since the latter ordinarily composes (as tetrabrom-kynurin ‖) by far the greater part of the precipitate formed.

Experiments on dogs. — For these experiments commercial cracker-dust containing as an average 1.46 per cent nitrogen was obtained in large quantity and kept in glass-stoppered bottles. This constituted the *carbohydrate* food fed. The *fat* used was a good quality of lard practically free from nitrogen. The other food-stuffs used will be referred to in the protocols.

The following experiments demonstrate the formation of kynurenic acid after the ingestion of various proteids of both vegetable and animal origin. The "dog biscuit" used was

---

* Cf. J. Bishop Tingle, American Chemical Journal, 1898, xx, p. 126.

† Capaldi, Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 92. Solomin (ibid., p. 498 note) recovered by this method 99 per cent of 0.210 gram kynurenic acid added to urine. The following figures show average duplicates obtained by us from a dog's urine containing small quantities: (a) 0.0852 gram, (b) 0.0872 gram.

‡ Jaffé, Zeitschr. f. physiol. Chemie, 1883, vii, p. 399.

§ Baumann, Zeitschr. f. physiol. Chemie, 1877, i, p. 62.

‖ Brieger, Zeitschr. f. physiol. Chemie, 1881, iv, p. 89.

a commercial preparation containing dried meat, carbohydrates (sugar-beet), etc.; the albumin was commercial albumen e sanguine ; the vegetable proteid was crystallized edestin (phytovitellin) prepared from hemp seed after the manner already described by one of us ; * the Witte's "pepton" was the widely used product made up almost entirely of proteoses (from fibrin). The latter preparation contained 14 per cent N. A mixture of inorganic salts as recommended by J. Munk † was daily added to the diet in experiment C.

Various investigators have demonstrated that the proteoses and peptones may show caloric and nutritive values equivalent to those of the proteids from which they originate.‡ Several of our experiments (B, C, D) show a characteristic excretion of kynurenic acid after repeated feeding of proteo' (Witte's "pepton"). No disturbances of the gastro-in nal tract (as with "Somatose," p. 235) were observed with th product.

* Chittenden and Mendel, Journal of Physiology, 1894, xvii, p. 49.
† J. Munk, Virchow's Arch. f. d. exper. Pathologie, cxxxii, p. 102.
‡ Cf. Munk and Ewald, Die Ernährung, 1895, p. 34.

DOG A.

Date. 1887.	Body Weight. kilos.	Urine. Vol. c.c.	Sp. Gr.	Reaction (Litmus).	Kynurenic Acid. grm.	Food.
Nov. 22	9.0	110	1026	Acid.	Trace.	Carbohyd.,* 50; fat, 50; water, 200.
23	8.9	165	1006	Acid.	None.	"
24	8.8	160	1011	Acid.	None.	"
25	8.8	195	1013	Acid.	None.	"
26	8.7	175	1016	Acid.	None.	"
27	8.7	190	1027	Alkaline.	0.012	Casein,* 50; carbohyd., 50; fat, 40; water, 200.
28	8.8	230	1012	Acid.	None.	Casein,† "
29	8.7	125	1013	Acid.	None.	Casein,† "
30	8.9	195	1024	Acid.	0.035	Casein, "
Dec. 1	8.9	165	1014	Acid.	None.	Casein,† 100 "
2	8.9	250	1015	Acid.	0.014	Casein,† "
3	8.8	70	1037	Acid.	0.036	Casein,† "
4	8.9	190	1046	Acid.	0.124	Meat, 300; little fat.
5	8.9	275	1045	Acid.	0.103	" 400; " "

Date						Food
Dec. 6	8.9	205	1048	Acid.	0.178	Meat, 400; little fat.
7	9.0	110	1047	Acid.	0.058	Carbohyd., 50; fat, 50; water, 200.
8	8.9	60	1041	Acid.	None.	" " " " 200.
9	8.9	90	1017	Acid.	None.	" " " " 200.
10	8.9	160	1017	Acid.	None.	Casein, 150; carbohyd., 25; fat, 12; water, 75.
11	8.9	320	1021	Acid.	0.023	" 240; " 50; " 25; " 100.
12	9.0	400	1017	Acid.	0.057	" 300; " 50; " 50; " 200.
13	9.0	160	1018	Acid.	0.032	" 75; " 25; " 25; " 100.
14	9.1	420	1018	Acid.	0.121	" 350; " 50; " 50; " 200.
15	9.1	65	1030	Acid.	0.017	Carbohyd., 50; fat, 50; water, 100.
16	9.1	85	1020	Acid.	0.005	" " " " 100.
17	9.1	120	1018	Acid.	None.	None (water).
18	8.9	85	1033	Acid.	None.	" "
19	8.8	70	1025	Acid.	None.	" "
20	8.6	65	1025	Acid.	None.	" "
21	8.7	50	1027	Acid.	None.	" "

*The casein in this and following experiments was either a commercial preparation, or casein prepared from milk, reprecipitated once and fed moist. The moist casein contained 25 to 30 per cent dry substance.

† Moist casein.

DOG B.

| Date. | Body Weight. | Urine. | | | | Food. |
1897.		Vol.	Sp. Gr.	Reaction (Latmus).	Kynurenic Acid.	grams.
	kilos.	c.c.			grm.	
Dec. 2	10.0	330	1012	Acid.	None.	None (water).
8	10.0	None.	:	:	:	" "
4	9.9	185	1024	Acid.	0.152	Meat, 800; little fat.
5	9.8	400	1044	Acid.	0.119	Meat, 400; little fat.
6	9.5	43	1048	Acid.	0.025	Liver, 150.
7	9.6	240	1048	Acid.	0.845	None (water).
8	:	None.	:	:	} 0.008*	" "
9	9.4	175	1089	Acid.	} 0.093	" "
10	9.0	None.	:	:	{ 0.097*	" "
11	:	None.	:	:	{ 0.097	" "
12	8.5	250	1034	Acid.	{ 0.097	" "
13	8.6	85	1034	Acid.	0.081	Carbohyd., 50; fat, 50; water, 200.
14	8.7	60	1048	Acid.	None.	" 100; " 100; " 300.

Dec. 15	8.7	225	1016	Acid.	None.	Carbohyd., 75; fat, 75; water, 150.
16	8.6	115	1024	Alkaline.	None.	Carbohyd., 25; fat, 25; water. "**Somatose**," 25.†
17	8.6	225	?	Acid.	None.	Carbohyd., 30; fat, 25; water. "**Somatose**," 50.
18	8.4	280	?	Alkaline.	None.	Carbohyd., 30; fat, 25; water. "**Somatose**," 50.
19	8.4	65	1024	Acid.	None.	"**Somatose**," 14, etc.
20	8.5	95	1024	Acid.	None.	Carbohyd., 25; water, 50.
21	8.5	100	1028	Acid.	None.	" 125; " 200.

* The figures given for the daily kynurenic acid output of this period are calculated from the total amount excreted, there being no urine voided on part of the days.

† "Somatose" is a commercial product prepared by digestion of meat and composed largely of proteoses (70 to 80 per cent) with small quantities of peptone (R. H. Chittenden, Dietetic and Hygienic Gazette, x, p. 47). The sample used contained 13.9 per cent N. In this animal it produced vomiting and diarrhœa, and doubtless only a portion of the material fed was absorbed. Irritation of the gastro-intestinal tract by large quantities of similar digestion products has frequently been observed. Cf. Munk u. Ewald, Die Ernährung des gesunden und kranken Menschen, 1895, p. 427; also Treupel, Münchner med. Wochenschr., 1898, p. 611.

DOG C.

Date. 1898.	Body Weight.	Urine.					Food.
	kilos.	Vol. c.c.	Sp. Gr.	Reaction (Litmus).	Nitrogen. grm.	Kynurenic Acid. grm.	grm.
Mar. 21	9.9	179	1063	Acid.	....	0.010	Dog biscuit.
22	9.9	222	1058	Acid.	....	0.036	"
23	9.9	None.	...	Acid.	....	....	"
24	10.1	380	1088	Acid.	12.08	0.011	"
25	10.0	460	1029	Alkaline.	8.59	0.099	Albumin, 50; carbohyd., 75; fat, 40.
26	10.0	455	1037	Acid.	11.68	0.168	" 75; "
27	10.0	325	1040	Acid.	8.53	0.187	" 100; "
28	10.0	400	1035	Acid.	10.27	0.106	" 100; "
29	9.9	174	1048	Acid.	5.00	0.053	" 50; "
30	10.0	430	1029	Acid.	13.42	0.125	" 50; "
31	10.0	554	1029	Acid.	17.94	0.141	Edestin, 75; "
April 1	9.9	385	1029	Acid.	13.08	0.187	" 100; "
2	10.1	415	1022	Acid.	9.32	0.191	" 100; "
3	10.1	350	1018	Acid.	6.69	0.009	Albumin, 15; "
4	10.0	222	1050	Acid.	9.61	0.018	" 15; "
5	10.0	276	1030	Alkaline.	8.17	0.428	Witte's "pepton," 75; carbohyd., 100; fat, 40.

DOG D.

Date. 1898.	Body Weight. kilos.	Urine. Vol. c. c.	Sp. Gr.	Reaction (Litmus.)	Kynurenic Acid. grm.	Food. grm.
April 17	8.8	202	1027	Acid.	0.028	Albumin, 15; carbohyd., 50; fat, 40; water.
18	8.8	105	1020	Acid.	Trace.	" " " "
19	8.3	180	1023	Acid.	None.	" " " "
20	8.3	150	1030	Acid.	0.017	" " " "
21	8.3	188	1041	Acid.	0.333	Albumin, 15; carbohyd., 50; fat, 40; Witte's "pepton," 75; water; daily.
22	8.4	312	1050	Acid.	0.276	
23	8.8	143	1055	Alkaline.	0.182	
24	8.6	550	1015	Neutral.	Trace.	Albumin, 15; carbohyd., 50; fat, 40; water.
25	8.5	264	1014	Alkaline.	None.	" " " "
26	8.4	258	1019	Acid.	None.	" " " "

DOG E.

Date. 1868.	Body Weight.	Urine.					Food.*
	kilos.	Vol. c.c.	Sp. Gr.	Reaction (litmus).	Nitrogen. grm.	Kynurenic Acid. grm.	grm.
May 20	9.9	366	1014	Acid.	5.02	0.044	Albumin, 15; carbohyd., 50; fat, 40; water.*
21	9.9	195	1014	Neutral.	2.05	0.039	" " " "
22	9.9	510	1011	Alkaline.	3.57	0.041	" " " "
23	9.8	500	1009	Neutral.	2.77	None.	" " " "
24	9.8	820	1016	Alkaline.	5.02	0.175	" 80; " " "
25	9.8	665	1016	Alkaline.	8.05	0.149	" " " "
26	9.8	660	1017	Alkaline.	6.83	0.200	" " " "
27	9.8	840	1012	Alkaline.	2.98	0.055	" 15; " " "
28	9.8	455	1010	Alkaline.	2.32	0.028	" " " "
29	9.7	410	1023	Alkaline.	7.48	0.064	" 40; gelatin, 40; carbohyd., 50; fat, 40; water.
30	9.5	440	1017	Alkaline.	1.47	None.	" " " "
31	9.5	560	1023	Alkaline.	12.17	0.108	" " " "
June 1	9.4	335	1011	Alkaline.	2.73	0.080	" 15; " " "
2	9.5	410	1015	Alkaline.	4.00	0.027	" 15; " " "
3	9.4	480	1017	Alkaline.	7.77	None.	Gelatin, 80; carbohyd., 50; fat, 40; water.
4	9.3	435	1019	Alkaline.	7.74	None.	" " " "
5	9.3	240	1024	Alkaline.	5.99	None.	" " " "
6	9.4	560	1009	Alkaline.	3.19	None.	Albumin, 15; " " "
7	9.5	350	1010	Alkaline.	1.84	None.	" 15; " " "
8	9.5	425	1014	Alkaline.	2.70	0.049	" 40; " " "
9	9.5	750	1010	Alkaline.	3.39	0.167	" 80; " " "

* Ad libitum.

The results obtained in the preceding experiment are summarized in the following table, which gives the daily averages for various feeding periods.

DOG E. — SUMMARY.

(Giving daily averages for various feeding periods.)

Date.	Urine.		Food.	
1898.	Nitrogen.	Kynurenic Acid.	Nitrogen.	Proteid.
	grm.	grm.	grm.	grm.
May 21–23	2.79	0.027	1.75	Albumin, 15.
24–26	6.63	0.175	9.36	" 80.
27, 28	2.65	0.044	1.75	" 15.
29–31	7.04	0.057	10.20	" 40; gelatin, 40.
June 1, 2	3.36	0.053	1.75	" 15.
3–5	7.17	None.	11.04	Gelatin, 80.
6, 7	2.51	None.	1.75	Albumin, 15.

In view of the peculiar chemical and physiological behavior of gelatin in contrast to the ordinary proteids, some experiments were undertaken with this albuminoid (Dogs F, G, H). Commercial gelatin, containing 13.8 per cent N, was fed, it being eagerly eaten when mixed with water and the other food stuffs as indicated. Occasionally 1 to 2 grams of Liebig's extract of beef were added to improve the flavor, while a mixture of inorganic salts as recommended by J. Munk [*] was daily given with the food. The following dog, F, which had some time previously been used in a phlorhizin experiment, had been fed very large quantities of casein on the days immediately preceding the experiment.

At the conclusion of the experiment on the dog H, the animal was starved for eighteen days. Body-weight fell from 7.6 kilos to 6.0 kilos. During this period 1220 c. c. of urine, containing 107 mgr. kynurenic acid, were eliminated.

[*] J. Munk, Virchow's Arch. f. d. exper. Pathologie, cxxxii, p. 102.

DOG F.

Date.	Body Weight.	Urine.					Food.*
1898.	kilos.	Vol.	Sp. Gr.	Reaction (Litmus).	Nitrogen.	Kynurenic Acid.	
		c.c.			grm.	grm.	
Feb. 1	7.2	188	1043	Acid.	6.11	0.073	Casein, 25; fat, 50; carbohyd., 50.
2	7.2	77	1032	Acid.	2.52	0.028	"　　"　　"　　"
3	7.2	248	1015	Acid.	8.51	None.	"　　"　　"　　"
4	7.1	112	1030	Acid.	3.79	None.	Gelatin, 35.
5	7.2	230	1027	Acid.	7.15	None.	"　40.
6	7.2	150	1028	Acid.	5.65	None.	"　45.
7	7.0	112	1022	Alkaline.	. .	0.008	Albumin, 20; carbohyd., 35.
8	7.0	155	1023	Alkaline.	8.72	0.046	"　30;　"　40;
9	7.0	228	1025	Alkaline.	7.23	0.060	"　40;　"　50;
10	7.0	477	1018	Alkaline.	3.28	0.104	"　50;　"　60;
11	7.1	260	1033	Alkaline.	10.16	0.138	"　60;　"　10.
12	7.0	248	1050	Alkaline.	8.02	0.076	"　60; gelatin, 35; carbohyd., 10.
13	6.9	108	1049	Alkaline.	9.57	0.003	"　50;　"　20;　"　35.
14	6.9	174	1044	Alkaline.	10.15	0.021	"　60;　"　"　"　"
15	6.9	480	1028	Alkaline.	9.71	0.128	"　50;　"　"　"　"
16	6.9	338	1020	Alkaline.	. .	0.072	"　60;　"　"　"　50.
17	6.9	294	1017	Alkaline.	. .	None.	"　40; fat, 40;　"　"
18	6.9	258	1026	Alkaline.	. .	0.079	"　30;　"　30;　"　"
19	7.0	455	1014	Neutral.	. .	None.	"　25;　"　40;　"　"
20	7.0	275	1016	Neutral.	. .	None.	"　"　"　"　"
21	7.0	298	1013	Alkaline.	. .	None.	"　"　"　"　"

* Water was given ad libitum.

DOG G.

Date. 1888.	Body Weight. kilos.	Urine. Vol. c.c.	Sp. Gr.	Reaction (Litmus).	Nitrogen. grm.	Kynurenic Acid. grm.	Food. grm.
Mar. 14	8.6	137	1046	Acid.	...	0.085	None; water.
15	8.3	None.	....	Acid.	...	0.069 ⎫	" "
16	8.3	150	1043	Acid.	...	0.069 ⎬ *	" "
17	8.0	70	1048	Acid.	...	0.069 ⎭	" "
18	8.0	211	1085	Acid.	...	0.137	Albumin, 20; carbohyd., 60; fat, 40; water.
19	8.0	170	1023	Neutral.	...	0.028	" " " "
20	8.0	320	1012	Acid.	...	0.028	" " " "
21	8.0	383	1015	Acid.	...	0.050	" " " "
22	8.0	214	1021	Acid.	...	0.068	" " " "
23	8.0	412	1017	Acid.	...	0.075	" " " "
24	8.0	345	1012	Acid.	2.47	0.016	" 75; " "
25	8.0	200	1018	Acid.	2.07	0.025	" 15; " "
26	8.0	146	1020	Acid.	1.79	0.028	" " " "
27	8.0	293	1021	Acid.	3.55	0.024	" " " "
28	8.0	275	1017	Acid.	8.18	0.011	" " " "
29	8.0	184	1054	Acid.	8.16	None.	15; Gelatin, 75; carbohyd., 75; fat, 40; water.
30	8.1	258	1041	Acid.	10.69	None.	" 100; " " "
31	8.2	624	1030	Acid.	19.66	None.	" " " " "
Apr. 1	8.0	548	1026	Acid.	12.15	0.015	" 50; " " "
2	8.1	290	1038	Acid.	5.96	0.053	Albumin, 100; carbohyd., 75; fat, 40; water.
3	8.0	265	1043	Acid.	7.61	0.091	" " " "
4	8.1	288	1018	Acid.	3.52	None.	Carbohyd., 75; fat, 40; water.
5	8.1	164	..	Acid.	1.67	None.	" " "

* The figures given for the daily Kynurenic acid output of this period are calculated from the total amount excreted, there being no urine voided on one of the days.

DOG H.

Date. 1898.	Body Weight. kilos.	Urine. Vol. c.c.	Sp. Gr.	Reaction (litmus).	Nitrogen. grm.	Kynurenic Acid. grm.	Food. grm.
Mar. 13	8.5	41	1033	Acid.	1.18	None.	None; water.
14	8.3	37	1031	Acid.	1.08	None.	" "
15	8.1	57	..	Acid.	1.81	0.012	" "
16	8.0	41	1033	Acid.	0.94	None.	" "
17	7.9	None.	..	..	1.35	0.015	" "
18	7.8	None.	..	..	1.35	0.015	" "
19	7.8	60	1050	Acid.	1.35	0.015	" "
20	7.6	160	1013	Acid.	1.87	0.010	" "
21	7.5	128	1019	Acid.	2.13	0.004	" "
22	7.6	105	1017	Acid.	1.53	0.017	Casein,‡ 100; fat, 100; water, 75.
23	7.6	94	1017	Acid.	1.56	0.016	" 50; " "
24	7.6	105	1023	Acid.	2.32	0.026	" "
25	7.7	138	1020	Acid.	2.46	0.018	" "

Mar. 26	7.8	56	1031	Acid.	1.47	0.011	Casein, ‡ 75; fat, 100; water, 75.
27	7.8	116	1028	Acid.	2.39	0.041	" " " " "
28	7.8	300	?	Acid.	..	None.	" gelatin, 35; fat, 100; water.
29	7.6	325	?	Acid.	8.01	None.	" " " " "
30	7.6	290	?	Alkaline.	..†	None.	" 85; " 70; " "
31	7.6	270	1030	Acid.	6.85	None.	" " " " "
April 1	7.7	162	1031	Acid.	5.53	0.061	" 350.
2	7.6	280	1035	Acid.	8.68	0.187	" 375.
3	7.6	330	1024	Acid.	8.81	0.028	" 350.
4	7.7	174	1044	Acid.	9.11	0.017	" 175; gelatin, 30.
5	7.6	150	1047	Acid.	8.27	None.	" 90; " 45.
6	7.6	75	1040	Acid.	8.97	None.	Fat, 100; " 15.
7	7.6	204	1022	Acid.	..	0.018	Casein, ‡ 215; carbohyd., 50; fat, 25; gelatin, 25.
8	7.7	295	1028	Acid.	..	0.084	" 300; gelatin, 30.

* Cf. note 1, Dog G.
† A small quantity of faeces became mixed with the urine and made an accurate urine-nitrogen determination impossible.
‡ Moist casein.

Discussion of the preceding analytical data. — So far as has been observed, all aromatic compounds found in the organism of the higher animals are derived either from benzene derivatives introduced into the system, or from the proteids, which must accordingly contain aromatic radicals. A synthesis of the latter in the animal body from carbohydrates or fats scarcely seems probable in view of the accumulated experimental data bearing on the problem.* The present study of kynurenic acid excretion lends additional force to this view as applied to quinoline derivatives. Thus kynurenic acid is almost always found in the urine during starvation, a condition in which body proteids form the source of the nitrogenous compounds excreted. This observation, repeatedly made (*e. g.*, Dogs G, H, J, K), leaves little doubt that kynurenic acid is a true product of proteid katabolism, and for the most part, at least, is not dependent for its origin on the putrefactive processes in the intestine, — a possibility which has already been mentioned.

Quantitatively considered, kynurenic acid production bears a more or less direct relation to the variations in the decomposition of proteid material, whether the latter be the " tissue proteid " of a starving animal, or introduced as food and the kynurenic acid formed incidental to its metamorphosis. Moreover, our experiments furnish repeated evidence that kynurenic acid is a concomitant or direct product of accelerated proteid metabolism. There is no lack of evidence that the proteid molecule may break down in the body into a nitrogenous and non-nitrogenous portion. The former part is doubtless rapidly further broken down, oxidized and synthesized perhaps into various nitrogenous constituents of the urine. The non-nitrogenous moiety is less speedily eliminated. It may be converted into glycogen, or dextrose, or fat; and forming, as it does, the major part of the original molecule, it may be distributed to the tissues more slowly in proportion as they demand it.† The experiments of Feder ‡

---

* Cf. Baumann, Zeitschr. f. physiol. Chemie, 1886, x, p. 123.

† Cf. Reilly, Nolan and Lusk, Amer. Jour. Physiol., 1898, i, p. 404.

‡ Feder, Zeitschrift für Biologie, 1881, xvii, p. 541.

and of Reilly, Nolan and Lusk * indicate that most of the nitrogen of ingested meat is, on the other hand, eliminated within a few hours. It may be imagined, then, that kynurenic acid is one of the nitrogenous products derived from this rapidly eliminated nitrogenous radical of the original proteid ; and it scarcely seems unreasonable to assume that this acid represents the metabolic end-product of quinoline-yielding radicals in the molecule.† In this connection it will be remembered that R. Cohn ‡ was able to obtain a pyridine derivative as a decomposition product of casein, thus demonstrating the possible presence of the pyridine ring in the constitution of the proteid molecule.

Granting conditions like those referred to, several probabilities are indicated. For example, it should follow that the extent of proteid katabolism going on should influence very largely the production of kynurenic acid. Our experiments with non-nitrogenous diet (fat and carbohydrate) are quite in harmony with this. Excessive proteid decomposition is avoided under such conditions, and kynurenic acid rapidly disappears from the urine. (Cf. Dog A, B.) Equally suggestive are the results obtained with a mixed diet containing only small amounts of proteid. The proteid-sparing effect of the other food-stuffs is here well brought out. In one animal (Dog A) an absence of kynurenic acid excretion during the first seven days of starvation was noted. The observation was an exceptional one, but perhaps not without significance, since the animal had an unusually well-nourished appearance, and the " fat " condition was remarkably persistent, even during the later days of starvation. We are inclined to attribute the results to the rather exceptional condition of the animal, since it is well known that the extent of nitrogenous katabolism in inanition is modified in a pronounced way by the relative as well as absolute amount of body fat in the individual.§

* Reilly, Nolan and Lusk, loc. cit., p. 404 fig.
† Cf. also Kretschy, Monatshefte für Chemie, 1881, ii, p. 85.
‡ R. Cohn, Zeitschr. f. physiol. Chemie, 1896, xxii, p. 171.
§ Cf. Munk and Ewald, Die Ernährung, 1895, pp. 22–23.

Kynurenic acid excretion has also been investigated in three dogs which were kept in nitrogenous equilibrium on a fixed diet of meat, fat, and carbohydrates. The animals were used in a research carried out in this laboratory on the influence of borax and boric acid on nutrition. Through the kindness of Dr. Gies we have obtained equivalent portions of each day's urine and have examined them for kynurenic acid, the fractions of each period being united and analyzed collectively. Further data are taken from the tables already published.*

FIRST EXPERIMENT.

Period.	Nitrogen Balance.	Uric Acid.	Kynurenic Acid.
9 days.	grm.	grm.	grm.
Fore	−0.981	0.428	None.
Borax (5 grams)	−1.928	0.411	0.101
After	−0.117	0.389	None.

SECOND EXPERIMENT.

10 days.			
Fore	+0.118	0.504	None.
Boric acid (1–2 grams) . .	−1.506	0.386	Trace.
After	−0.980	0.508	None.

THIRD EXPERIMENT.

8 days.			
Normal	+0.429	0.315	None.
Borax (2–5 grams) . . .	−0.801	0.293	None.
After	+0.661	0.248	None.
Boric acid (1–3 grams) . .	+2.174	0.310	None.
After	+2.122	0.354	None.
Borax (8 grams)	**−4.878**	**0.295**	**0.414**
After	−0.661	0.328	None.

It will be observed from the tables that the only periods during which kynurenic acid appeared in the urine were those in which administration of borax or boric acid gave rise to a direct stimulation of proteid metabolism. This is particularly brought out in the third experiment, during which a daily

* Chittenden and Gies, Amer. Journ. Physiol., 1898, i, p. 1.

dose of eight grams of borax produced a nitrogen deficit of over four grams. Uric acid, however, was continually present in the urine of the dogs employed; and the results obtained are in harmony with the opinion that kynurenic acid excretion is a phenomenon accompanying pronounced stimulation of proteid katabolism rather than ordinary conditions of body equilibrium.

The gelatin experiments. — Eckhard [*] stated that after feeding gelatin to a dog he failed to find kynurenic acid in the urine. Rosenhain [†] likewise obtained no kynurenic acid after feeding two and a half pounds of gelatin and six pounds of bread to a large dog in the course of a week. After a meal of one kilo of meat, however, the same animal excreted as much as 0.995 gram of kynurenic acid during the succeeding twenty-four hours. No data are presented to show that the gelatin was absorbed satisfactorily.

The results obtained with gelatin feeding in the present investigation afford an interesting confirmation of previous observations. The experiments of J. Munk [‡] have shown that in dogs over two-thirds of the required proteid of the diet may be replaced by gelatin with maintenance of nitrogenous equilibrium. Gelatin is, as a rule, readily digested and burned in the body, and has a pronounced proteid-sparing action like a typical non-proteid food. Lusk and his co-workers have recently shown that gelatin — like the proteids proper — may yield 60 per cent of sugar in the metabolic changes it undergoes in the body, as was evidenced by the amount of sugar excreted when the albuminoid was fed to a fasting animal in phlorhizin diabetes. Gelatin differs chemically from the ordinary proteids, in that tyrosin has not been found among its decomposition products.[§] The absence of tyrosin

[*] Eckhard, Ann. Chem. Pharm., 1856, xcvii, p. 358.

[†] Rosenhain, Beiträge zur Kenntniss der Kynurensäurebildung im Thierkörper. Inaugural-Dissertation. Königsberg, 1886, p. 8.

[‡] J. Munk, Arch. f. d. ges. Physiol., 1894, lviii, p. 309.

[§] Halliburton, Schaefer's Physiology, 1898, i, p. 71. It may be added that K. B. Lehmann failed to accomplish proteid synthesis in rats by feeding them

DOG J.

Date. 1898.	Body Weight. kilos.	Urine Vol. c.c.	Sp. Gr.	Reaction (Litmus).	Nitrogen. grm.	Kynurenic Acid. grm.	Dextrose. grm.	Food. grm.
Jan. 11	8.8	197	1029	Acid.	3.90	0.023	...	None.
12	8.5	:	:	:	:	{ 0.008*	...	"
13	8.3	96	1033	Acid.	2.54	{ 0.008*	...	"
14	8.1	120	1034	Acid.	3.64	0.078	...	" ; water.
15	8.0	:	:	:	:	{ 0.070*	...	"
16	7.9	142	1030	Acid.	4.91	{ 0.070*	...	"
17	7.7	78	1044	Acid.	2.04	0.060	...	Phlorhizin 1 (three times a day).
18	7.5	172	1056	Acid.	4.57	0.130	10.49	"
19	7.5	300	1058	Acid.	8.18	0.168	26.67	"
20	7.3	380	1056	Acid.	7.88	0.141	25.08	Casein, 100 ; fat, 100 ; water, 75.
21	7.2	226	1063	Acid.	6.27	0.160	19.06	" 50 ; " " "
22	7.0	176	1037	Acid.	8.82	0.063	...	" 50 ; " " "
23	6.9	98	1025	Acid.	2.04	0.029	...	" 50 ; fat, 100 ; water, 75.
24	6.9	112	1017	Acid.	1.78	0.059	...	" 50 ; " " "
25	6.9	134	1016	Acid.	1.56	0.030	...	" 75 ; " 150 ; " 100.
26	7.0	76	1018	Acid.	1.22	0.006	...	" 25 ; " 50 ; " 100.
27	6.9	127	1021	Acid.	2.11	None.	...	" 820 ; water.
28	7.0	265	1023	Acid.	3.48	0.082	...	" 545 ; "
29	6.9	370	1016	Acid.	6.96	0.091	...	" 574 ; "
30	7.1	295	1021	Acid.	7.83	0.122	...	" 515 ; "
31	7.2	385	1026	Acid.	12.24	0.189	...	

* Daily average calculated from two days' urine.

is suggestive of a deficiency in aromatic groups in the molecule, and it is not unlikely that this fact may account for the absence of the related compound kynurenic acid (oxyquinoline-carboxylic acid) when gelatin exclusively is fed. (Cf. Dogs E, F.) Out of 905 grams gelatin fed to small dogs in fourteen days we have obtained only 0.015 gram kynurenic acid, and that on a single day. Moreover, the nitrogen determinations in the urine (cf. Dogs E, F, G, H) give evidence of a ready absorption of the gelatin ingested; while the experiments, including simultaneous feeding of typical proteids like casein and albumin with the gelatin, show that the latter exercises no

DOG J. — SUMMARY.

Period.	Urine.	
	Nitrogen.	Kynurenic Acid.
	grm.	grm.
Fasting, 3 days . . .	7.50	0.200
Phlorhizin, 3 days . .	20.63	0.440
Casein, etc., 3 days . .	12.13	0.243

specific action in preventing kynurenic acid excretion when sufficient proteid is given along with it. Indeed, the behavior of gelatin precisely resembles that of the carbohydrates and fats. These experiments considered in connection with Lusk's observations indicate that the essential physiological peculiarities of gelatin are to be sought in the chemical structure of the *nitrogenous* portion of the molecule; and the failure of the albuminoid to give rise to kynurenic acid in the dog is doubtless associated with the lack of certain aromatic radicals in its make-up. Lastly, attention is directed to the ready assimilation of crystallized vegetable proteid (Dog C), and to the evidence

with gelatin and tyrosin (Sitzungsber. d. morphol.-physiol. Gesellsch. in München, 10 März, 1885). Contrary to the current statements, cf. Neumeister, Lehrbuch der physiol. Chemie, 1897, p. 63, pure gelatin gives a reaction with Millon's reagent which may perhaps be due to a far smaller proportion of aromatic radicals than is present in ordinary proteids. See Van Name, Journal of Experimental Medicine, 1897, ii, p. 128.

offered of the close physiological relationship between the animal and vegetable products, despite minor differences in chemical structure.[*]

Phlorhizin experiments.—In confirmation of the view that kynurenic acid excretion is incidental to excessive proteid katabolism, additional experiments on animals suffering from phlorhizin diabetes are presented. It has been shown that under these conditions sugar production goes on in the fasting animal directly at the expense of tissue proteid, the amount of nitrogen in the urine going parallel with the amount of sugar excreted; [†] and the sugar excretion may thus go on even in the absence of glycogen in the liver.[‡] Lusk [§] and his co-workers have shown that in the fasting dog a constant ratio of dextrose to nitrogen excreted is maintained during phlorhizin diabetes (D: N : : 3.75 : 1), and that feeding meat or gelatin does not change the ratio. The figures indicate a production of about 60 grams of dextrose from 100 grams of proteid, and it has furthermore been found that in this form of diabetes the proteid metabolism may increase to an extent as high as 560 per cent above that in simple inanition. Tables J and K show the extent of kynurenic acid production observed on two fasting dogs during phlorhizin diabetes. The phlorhizin, dissolved in dilute sodium carbonate solution, was introduced subcutaneously about every eight hours. Water was given ad libitum.

Here again the production of kynurenic acid during fasting is observed. Coincident with the appearance of the sugar in the urine occurs a rise in nitrogen excretion accompanied likewise by an increase in kynurenic acid. The figures become more striking when the daily averages of the period preceding and succeeding the phlorhizin days are presented in contrast.

---

[*] Cf. Rutgers, Zeitschrift für Biologie, 1888, xxiv, p. 351.

[†] Cf. for example, Cremer u. Ritter, Zeitschrift für Biologie, 1893, xxix, p. 256.

[‡] Thiel, Arch. f. exper. Pathol. and Pharmakol, 1887, xxiii, p. 142. Cf. also Hédon, Comptes rendus de la Soc. de Biologie, Paris, 1897, (10), iv, p. 60.

[§] Reilly, Nolan, and Lusk, Amer. Journ. Physiol., 1898, i, p. 395.

DOG K.

Date. 1898.	Body Weight. kilos.	Urine. Vol. c.c.	Sp. Gr.	Reaction (Litmus).	Nitrogen. grm.	Kynurenic Acid. grm.	Dextrose. grm.	Food. grm.
May 19	12.4	. .	. .	. .	4.21	0.178	. .	None; water.
20	12.3	244	1042	Acid.	4.21	0.178	. .	"
21	12.2	. .	. .	. .	2.42	0.065	. .	"
22	12.0	. .	. .	. .	2.42	0.065	. .	"
23	11.8	. .	. .	. .	2.42	0.065	. .	"
24	11.8	280	1046	Acid.	2.42	0.065	. .	"
25	11.8	290	1058	Acid.	9.00	0.208	39.15	Phlorhizin, 1; three times a day.
26	11.6	420	1055	Acid.	8.82	0.255	29.82	" 1; twice a day.
27	. .	520	1048	Acid.	10.86	0.342	85.88	None; water.
28	9.9	325	1061	Acid.	7.85	0.172	22.42	"
29	9.6	210	1050	Acid.	. . *	0.073	11.55	"
30	9.4	265	?	Acid.	4.38	0.053	12.98	"
31	9.2	405	1025	Acid.	2.47	None.	. .	"
June 1	9.3	180	1020	Acid.	. .	None.	. .	"

* Faeces in the urine.

The increased output of kynurenic acid attending the large increase in proteid katabolism (over that in inanition) thus justifies the emphasis placed upon the close relationship between these factors.   Owing to less careful conditions of experiment (lack of catheterization, etc.) the ratio of dextrose to nitrogen is somewhat lower than that found by Lusk.   In
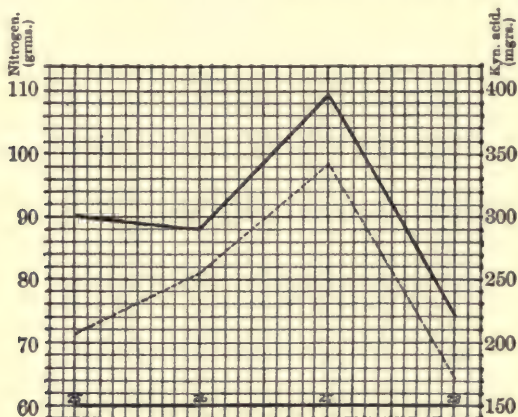
FIGURE 1.

Dog K.   The abscissæ represent the successive days of the experiment; the ordinates of the unbroken line represent grams of N excreted, those of the broken line represent milligrams of kynurenic acid.

the experiment on Dog K the ratio is higher.   In this animal sugar persisted in the urine for several days after the phlorhizin injection.   The ratio of dextrose to nitrogen during the phlorhizin days was as 3.68 : 1.   The other phenomena observed resemble those of the preceding experiment, namely, increased kynurenic acid excretion accompanying stimulated proteid decomposition,* as shown in the curves of Fig. 1.

Amyl nitrite experiment. — The production of diabetes in the dog by means of amyl nitrite has been demonstrated by a number of investigators.† We have also studied the action of

---

* In the case of one dog which excreted no kynurenic acid during six days' starvation we were unable to get similar results with phlorhizin.

† Araki, Zeitschr. f. physiol. Chemie, 1891, xv, p. 558. The older literature is referred to here.

this drug in the case of a dog. The 10-kilo animal had been fed three days on a diet consisting of albumin, 15 grams; carbohydrate, 50 grams; fat, 40 grams; — under which conditions nothing more than traces of kynurenic acid were found in the urine. On the fourth day the animal received three subcutaneous injections of amyl nitrite (about 6 c.c. being given in all). By the following day the dog was dead. The urine (128 c.c.) removed from the bladder contained no kynurenic acid; 4.69 grams sugar were present, and the ratio of dextrose to nitrogen was found as 7.4 : 1. Traces of albumin were also found, in agreement with observations of Araki.* The high ratio of dextrose to nitrogen indicates that the sugar found could not have its origin wholly in proteid decomposition, nor was this to be expected under these conditions (previous feeding, etc.). But Thiel† has shown that amyl nitrite fails to produce characteristic glycosuria in hens, although phlorhizin is effective in these animals. Amyl nitrite diabetes is therefore doubtless of a distinctly different type from that produced by phlorhizin; at any rate the absence of kynurenic acid in the urine in our single experiment corresponds with the slight evidence of proteid katabolism obtained.

Experiments on other animals.— So far as we are aware, no one has succeeded in finding kynurenic acid in the urine of any animal other than the dog. Hofmeister,‡ after careful examination, failed to find it in human urine. It has likewise been found missing in the urine of the rabbit,§ wolf,‖ and fox.‖ We have searched for kynurenic acid in the urine of the cat during inanition as well as during meat and milk diet, but always with negative results. In view of the evidence of our

---

* Araki, Zeitschr. f. physiol. Chemie, 1891, xv, p. 553. The older literature is referred to here.

† Thiel, Arch. f. exper. Pathol. u. Pharmakol., 1887, xxiii, p. 142.

‡ Hofmeister, Zeitschr. f. physiol. Chemie, 1881, v, p. 69. Eckhard, long before, had missed it in human urine; see Ann. Chem. Pharm., 1856, xcvii, p. 358.

§ Schmidt, Ueber das Verhalten einiger Chinolinderivate im Thierkörper, mit Rücksicht auf die Bildung von Kynurensäure. Inaugural-Dissertation, Königsberg, 1884.

‖ Capaldi, Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 87.

experiments regarding the formation of kynurenic acid in the dog from body proteids during inanition, several rabbits were starved for varying periods (three to five days) and the urine then examined. No kynurenic acid was found. Lastly the urine of man has been examined in wasting diseases (severe diabetes) without avail. Since Hauser [*] and Solomin [†] have observed that kynurenic acid introduced into the organism of man, rabbit, or dog, is in good part destroyed (especially in the case of the first two), it may be, as Solomin suggests, that kynurenic acid is absent from human and rabbit's urine not because it fails to be produced, but rather because it is destroyed in these organisms as rapidly as it is formed.

Theoretical considerations regarding the quantity of kynurenic acid obtainable. — That the absolute amount of kynurenic acid produced at any time should be large is scarcely to be expected. If, as the experiments of Lusk and his co-workers have indicated, the proteid molecule yields on cleavage in the body an amount of sugar equal to nearly 60 per cent, there remains " a nitrogen-containing radical in which the carbon and nitrogen would appear in the atomic ratio of 2.2 of C to 1 of N." [‡] Now kynurenic acid, $C_{10}H_7NO_3$, contains 10 of C to 1 of N; obviously the nitrogenous proteid radicals could not yield large quantities of a body of the composition indicated.

Summary. — Kynurenic acid is a direct product of proteid katabolism, and, as Baumann's experiments indicated, does not owe its immediate origin to putrefactive changes in the intestine.

Kynurenic acid excretion accompanies accelerated proteid decomposition, whether this condition be brought about by starvation, ingestion of large amounts of proteid food, or through the action of drugs (borax, phlorhizin).

Similar results follow the ingestion of both animal and vegetable proteids, as well as proteoses; gelatin, however,

[*] Hauser, Arch. f. exper. Pathol. u. Pharmakol., 1895, xxxvi, p. 1.

[†] Solomin, Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 497.

[‡] Reilly, Nolan, and Lusk, Amer. Jour. Physiol., 1898, i, p. 409.

does not give rise to kynurenic acid in metabolism, acting precisely like the carbohydrates in this respect. In conditions of ordinary nitrogenous equilibrium, or under the influence of proteid-sparing foods, kynurenic acid excretion is greatly diminished or absent.

The observations suggest the presence of quinoline-like radicals in the proteid molecule; the existence of a large carbohydrate group is also confirmed.

Uric acid and kynurenic acid may occur together in dog's urine, as Solomin has found. Kynurenic acid is absent from the urine of the cat during fasting and proteid feeding, and is not found in the urine of the rabbit during inanition.

VARIATIONS IN THE AMYLOLYTIC POWER AND CHEMICAL COMPOSITION OF HUMAN MIXED SALIVA.[*]

By R. H. CHITTENDEN and A. N. RICHARDS.

Since saliva is the product of secretory glands having their periods of comparative rest and activity, it follows quite naturally that this secretion might be expected to show variations in amylolytic power at different periods of the day: *i. e.*, that the secretion obtained after a period of glandular activity might possess less starch-digesting power than the secretion coming from glands which have been in a state of rest — due mainly to variations in the proportion of active enzyme present. Further, the well-known sensitiveness of the amylolytic enzyme to changes of reaction suggests also the possibility of fluctuations in amylolytic power dependent primarily upon changes in the proportion of alkaline-reacting salts contained in the secretion. In spite of the large amount of work of a chemico-physiological nature done upon saliva, these questions have received very little attention. During the past year, however, Hofbauer,[†] in an interesting communication has presented a series of results, bearing on the daily fluctuations in the amylolytic power of saliva, but his observations were limited solely to determination of the starch-digesting power at different periods of the day without regard to any possible relationship between the amylolytic power and the chemical composition of the secretion. His results, however, show

clearly that human mixed saliva does fluctuate in amylolytic power throughout the twenty-four hours, and further that the starch-digesting power of the saliva secreted before breakfast, for example, is greater than that of the secretion collected after breakfast. Our results afford distinct confirmation of the general truth of this observation. Hofbauer states in his paper that the only previous work bearing upon this subject is that by Chittenden and Ely.* The latter work, however, has no bearing whatever upon the question of possible variation in the amylolytic power of the secretion at different periods of the day. Indeed, in the paper in question it is distinctly stated that " the saliva was collected generally an hour or two after breakfast," with the distinct object of avoiding possible variations in composition due to the period of collection. The sole object of that investigation was to ascertain whether there is any connection between possible variations of alkalinity and the amylolytic power of saliva. The results there reported afford no indication whatever of the relative amylolytic action of the secretion for different periods of the day, since the fluids studied were invariably collected at essentially the same hour. It was ascertained, however, that the alkalinity of mixed saliva as measured by titration with a standard acid, using cochineal as an indicator, was fairly constant for a given individual at a given period of the day (9–10 A.M.), while saliva from different individuals may show a constant difference in alkalinity, although in the majority of cases the alkalinity varied only within narrow limits. In amylolytic action, however, there were no corresponding differences; fluctuations were observed, but within too narrow limits to indicate any tangible relation between the two factors.

It has become the custom to assume that the alkalinity of saliva, as indicated by its reaction toward litmus paper, is due more or less to the presence of sodium carbonate. Thus, in the latest text-book of physiology the statement † is made

* Chittenden and Ely, Amer. Chem. Journ., 1883, iv, p. 829.
† Text-book of Physiology, edited by E. A. Schäfer, 1898, i, p. 504.

that "the alkalinity of saliva depends upon the presence of sodium carbonate. In man and in the dog the percentage of this salt varies from 0.08 to 0.19 per cent." So far as we are aware, however, there is no justification for this statement. In the earlier work from this laboratory * it was stated that the average alkalinity for fifty-one samples of human mixed saliva was 0.08 per cent, "expressed in the form of sodium carbonate." Further, in all the tabulated results contained in that paper, the alkalinity, as measured by titration with standard acid in the presence of cochineal as an indicator, was carefully expressed as " equivalent in Na_2CO_3," this being done to avoid any positive statement as to the exact cause of the alkalinity. Further, in the oft-quoted work of Werther † the alkalinity of the saliva of the dog was determined by titration with decinormal sulphuric acid with litmus as an indicator: a method which obviously would throw no light upon the cause of the alkalinity. Moreover, in at least some of the tables containing his results the percentage of alkalinity is expressed as "alkalinity calculated as Na_2CO_3."

Examination of a large number of samples of human mixed saliva obtained from different individuals at different periods of the day convinces us that, under normal conditions at least, human saliva never contains the least trace of sodium carbonate. Toward litmus, lacmoid, etc., human saliva constantly reacts alkaline, but with phenolphthaleïn it invariably shows an acid reaction, and a certain amount of a decinormal alkali solution is required to bring out an alkaline reaction with this indicator. Further, phenolphthaleïn is an extremely sensitive reagent for sodium carbonate; a solution containing 0.001 per cent of sodium carbonate will give a pink color when brought in contact with a solution of phenolphthaleïn. With human saliva, however, we have never obtained any color reaction with phenolphthaleïn whatever; the solution invariably remains colorless, thus proving that the alkalinity indicated by litmus must be due to some acid salt or salts, like the hydro-

---

* Chittenden and Ely, Amer. Chem. Journ., 1883, iv, p. 333.
† Werther, Archiv f. d. ges. Physiol., 1886, xxxviii, p. 293.

gen alkali phosphates, with possibly some alkali bicarbonate. The submaxillary saliva of the dog, however, obtained by stimulation of the chorda tympani is usually, at least, faintly alkaline to phenolphthaleïn; * consequently this fluid may owe its alkalinity in part to sodium carbonate. These facts, which admit of easy confirmation, are worthy of some consideration, since they have an important bearing upon the normal conditions governing enzyme action.

I. RELATIVE ALKALINITY AND ACIDITY OF HUMAN SALIVA BEFORE AND AFTER EATING.

In this series of experiments the saliva was collected from one individual, stimulation of the secretion being effected by chewing a small piece of rubber. About 15 c.c. of fluid were collected each time. The portion collected before breakfast was obtained at 7.30 A. M., half an hour before eating, while the portion collected after eating was obtained fifteen minutes after the close of the meal. The alkalinity was determined by titrating the saliva (5 c.c.) with a decinormal solution of sulphuric acid, using lacmoid as an indicator, while the acidity was determined by the use of a decinormal solution of sodium hydroxide, the indicator being phenolphthaleïn. The alkalinity was calculated in terms of sodium carbonate, and is also expressed as milligrams of H_2SO_4 (absolute) required to neutralize 1 gram of saliva. The degree of acidity is expressed as milligrams of NaOH (absolute) required to neutralize 1 gram of saliva. Following are the results obtained (see table, p. 260).

A glance at these results shows that the alkalinity of saliva, as indicated by lacmoid, is noticeably greater in most cases in the fluid secreted after a night's rest, before breakfast, than in the secretion obtained after the glandular activity induced by the morning meal. Before and after dinner, however (1 P. M.), this distinction is less conspicuous. It is also interesting to note that the average alkalinity, expressed in terms of sodium carbonate, is somewhat higher with lacmoid as an indicator

---

* Chittenden, Science, n. s., 1897, v, p. 902. Also Chittenden, Mendel, and Jackson, Amer. Jour. Physiol., 1898, i, p. 174.

Time.	Alkalinity.		Acidity.
	Expressed as Na_2CO_3.	Milligrams H_2SO_4 to Neutralize 1 gram Saliva.	Milligrams NaOH to Neutralize 1 gram Saliva.
	per cent.		
Before Breakfast .	0.163	0.78	0.11
After Breakfast .	0.127	0.61	0.04
Before Breakfast .	0.193	0.98	0.06
After Breakfast . .	0.130	0.64	0.08
Before Breakfast .	0.142	0.69	0.06
After Breakfast . .	0.122	0.59	0.06
Before Breakfast .	0.132	0.64	0.10
After Breakfast . .	0.132	0.64	0.08
Before Breakfast .	0.173	0.88	0.08
After Breakfast . .	0.127	0.61	0.04
Before Breakfast .	0.148	0.71	0.11
After Breakfast . .	0.132	0.64	0.08
Before Breakfast .	0.168	0.81	. . .
After Breakfast . .	0.127	0.61	. . .
Before Breakfast .	0.122	0.59	0.11
After Breakfast . .	0.122	0.59	0.08
Before Breakfast .	0.148	0.71	. . .
After Breakfast . .	0.137	0.66	. . .
Before Dinner . .	0.132	0.64	0.08
After Dinner . . .	0.142	0.69	0.02
Before Dinner . .	0.168	0.81	0.08
After Dinner . . .	0.158	0.76	0.08
Before Dinner . .	0.153	0.73	0.06
After Dinner . . .	0.158	0.76	0.02

than with litmus or cochineal; a fact which would be expected in view of the presence of the hydrogen alkali phosphates contained in the fluid. It is likewise to be seen that the average acidity as indicated by phenolphthaleïn, though less conspicuous, is also inclined to diminish after eating.

II. RELATIVE ALKALINITY AND AMYLOLYTIC POWER OF HUMAN SALIVA BEFORE AND AFTER EATING.

In this series of experiments the main object was to ascertain whether there are noticeable variations in the amylolytic power of saliva before and after eating and whether such variations, if existent, run parallel with variations in the alkalinity. As in the previous experiments, the saliva was collected by chewing a small piece of rubber.

Amylolytic power was determined as follows: 5 c.c. of the filtered saliva were diluted with distilled water to 50 c.c.; 10 c.c. of the diluted fluid were then added to 1 gram of pure arrowroot starch made into a paste with 90 c.c. of water, and the mixture kept at 38° C. for half an hour. Amylolysis was then stopped by boiling the fluid, after which the solution, when cool, was made up to 150 c.c. with water and the reducing sugar determined by the Allihn Method, using 25 c.c. of the sugar-containing solution. The results are expressed as milligrams of maltose formed from 1 gram of starch by 1 c.c. of saliva. The data obtained are given below.

Collector.	Time.	Alkalinity.		Amylolytic Power.
		Expressed as Na_2CO_3.	Milligrams H_2SO_4 to Neutralize 1 gram Saliva.	Milligrams Maltose formed by 1 c.c. Saliva.
		per cent.		
R.	Before Breakfast	0.173	0.83	523.4
	After Breakfast	0.127	0.61	511.8
R.	Before Breakfast	0.168	0.81	630.6
	After Breakfast	0.127	0.61	588.8
R.	Before Breakfast	0.148	0.71	562.2
	After Breakfast	0.132	0.64	485.4
R.	Before Breakfast	0.122	0.59	620.4
	After Breakfast	0.122	0.59	534.6
R.	Before Breakfast	0.148	0.71	549.0
	After Breakfast	0.137	0.66	510.5
J.	Before Breakfast	. . .	. . .	209.4
	After Breakfast	. . .	. . .	224.4
M.	Before Breakfast	0.117	0.56	585.0
	After Breakfast	0.102	0.49	468.6
M.	Before Breakfast	. . .	. . .	621.0
	After Breakfast	. . .	. . .	587.6
R.	Before Dinner	0.153	0.73	549.6
	After Dinner	0.158	0.76	536.4
R.	Before Dinner	0.142	0.69	582.0
	After Dinner	0.142	0.69	564.6
R.	Before Dinner	. . .	. . .	570.0
	After Dinner	. . .	. . .	562.8
R.	Before Dinner	0.163	0.78	599.8
	After Dinner	0.158	0.76	606.6
R.	Before Dinner	. . . .	. . .	594.0
	After Dinner	. . . .	. . .	547.2

From these results it would appear that saliva secreted after a period of glandular inactivity, as before breakfast, is ordinarily possessed of greater amylolytic power than the secretion obtained after eating; results which accord closely with Hofbauer's observations.   Before and after dinner (1 P. M.), however, the difference in amylolytic power is less pronounced; a fact which might be expected in view of the short period for recuperation between the breakfast and dinner and because of the more or less constant stimulation of the salivary glands during the waking hours.   Further, we see in these results a suggestion of some degree of relationship between the percentage of alkaline salts contained in the saliva and its amylolytic power.   Before breakfast, for example, the content of alkaline salts and the starch-digesting power of the secretion are greater than in the fluid secreted after glandular activity.   At first glance, then, it might seem that the variations in amylolytic action noticed above are due to changes in the proportion of alkaline salts.   The objection to this view, however, is that it associates the higher degree of amylolytic power with the higher percentage of alkalinity, whereas numerous trustworthy experiments tend to show that saliva manifests its highest degree of digestive power in a perfectly neutral fluid.*   Consequently, if the above variations in amylolytic action are primarily due to changes in the proportion of alkaline-reacting salts, then the higher degree of amylolysis should be connected with the lower degree of alkalinity.   As the reverse is true, the more plausible and natural explanation of the results is that the higher degree of amylolysis is connected primarily with the presence of larger amounts of the amylolytic enzyme, and as this is presumably connected with the outpouring of a more concentrated secretion a corresponding increase in alkaline-reacting salts might naturally be expected.   Further, in harmony with the latter view it is to be noticed that the secretions obtained before and

* Langley and Eves, Journal of Physiology, 1883, iv, p. 18. Chittenden and Smith, Studies in Physiological Chemistry, Yale University, 1885, i, p. 8.

after breakfast fail to show any close parallelism between the variations in amylolytic power and variations in alkalinity. Thus, the most marked differences in digestive power are frequently seen with salivas which show only a slight difference in alkalinity, and on the other hand marked differences in alkalinity may be associated with minor differences in amylolytic power.

III. Alkalinity, Amylolytic Power and Composition of Human Saliva before and after Eatng.

In view of the preceding results, the following set of experiments was tried, in which, in addition to alkalinity and amylolytic power, the proportion of dry solids and inorganic salts of the saliva was likewise determined. The dry solids were determined by simply drying a weighed amount of the filtered saliva — usually 5 grams — on a water-bath and heating at 105° C. in an air-bath until of constant weight. The inorganic salts were then determined by careful ignition of the residue. In some of the following experiments relative amylolytic action was determined by Robert's * method, the method being based on the different lengths of time which solutions of different amylolytic power require to digest a certain amount of starch paste to the achromic point. The results obtained by this method are expressed in minutes; *i. e.*, the number of minutes which elapse from the time the diluted saliva is added to the starch paste until the appearance of the achromic point.

The results obtained are shown in the table on p. 264.

In these results we have a suggestion of the same general tendency toward decrease of amylolytic power and lowered content of alkaline salts in the saliva secreted after the morning meal; while as accompanying results we see corresponding fluctuations (although not in all cases) in the proportion of total solids, organic matter, and inorganic salts, thus bearing out the view that the variations in amylolytic power are connected mainly with changes in the general concentration

* See Gamgee's Physiological Chemistry of the Animal Body, ii, p. 56.

Collector.	Time. Breakfast.	Vol.	Alkalinity.		Amylolytic Power.	Total Solids.	Organic matter.	Inorganic Salts.
			AsNa₂CO₃.	Mg . H₂SO₄ to neutralize 1 gram Saliva.	Mg. Maltose formed by 1 c.c. Saliva.			
		c.c.	per cent.			per cent.	per cent.	per cent.
R.	Before	30	0.158	0.76	649.2	1.02	0.77	0.24
	After	25	0.122	0.59	601.2	0.51	0.33	0.17
R.	Before	25	0.163	0.78	651.0	0.86	0.58	0.28
	After	40	0.112	0.55	615.6	0.51	0.30	0.21
R.	Before	20	0.122	0.59	467.4	0.44	0.23	0.22
	After	25	0.102	0.49	491.4	0.40	0.19	0.21
D.	Before	20	0.081	0.39	43.0 *	0.37	0.21	0.16
	After	20	0.096	0.46	50.0	0.39	0.24	0.15
A.	Before	50	0.153	0.73	12.0	0.45	0.30	0.15
	After	40	0.158	0.76	15.0	0.53	0.34	0.19
B.	Before	40	0.137	0.66	13.0	0.32	0.15	0.17
	After	30	0.132	0.64	8.0	0.37	0.21	0.16

of the secretion. At the same time it is to be observed that the above differences in composition and amylolytic power are much more marked with the individual R than with A, B, and D. In fact, with the latter three individuals there is very little difference in composition in the saliva before and after the morning meal, and further in the third experiment with R the amylolytic power *after* the meal is greater than that of the saliva secreted before eating. These results have led to another series of experiments having in view especially the determination of the fluctuations in the character of the saliva throughout the day.

IV. VARIATIONS IN THE COMPOSITION AND AMYLOLYTIC POWER OF HUMAN SALIVA THROUGHOUT THE DAY.

In the first series of experiments under this head the saliva studied was collected by chewing a piece of rubber. The midday dinner was omitted; breakfast, however, was taken at 7.50 A. M. and supper at 6.40 P. M. Samples of saliva

---

* In this and the two following experiments amylolytic power was determined by Robert's method.

were analyzed every hour or two throughout the day. Following are the results obtained: —

Date.	Time.	Volume Saliva.	Alkalinity Calculated as Na_2CO_3.	Amylolytic power. Milligrams Maltose.	Total Solids.	Organic Matter.	Inorganic Salts.
	A.M.	c.c.	per cent.		per cent.	per cent.	per cent.
Jan. 20	7.15 to 7.30	21	0.112	574.2	0.59	0.29	0.30
20	7.50 to 8.15, Breakfast						
20	8.40 to 8.55	25	0.091	469.2	0.41	0.18	0.23
20	10.00 to 10.15	29	0.102	544.8	0.44	0.24	0.20
20	11.00 to 11.18	28	0.102	517.2	0.40	0.23	0.17
	P.M.						
20	12.00 to 12.13	21	0.132	183.6	0.39	0.16	0.23
20	12.45 to 12.55	19	0.112	280.8	0.40	0.20	0.20
20	2.00 to 2.15	21	0.112	270.6	0.37	0.16	0.21
20	3.00 to 3.12	21	0.132	217.8	0.44	0.26	0.18
20	4.00 to 4.13	22	0.132	382.8	0.47	0.27	0.20
20	5.00 to 5.14	23	0.153	575.4	0.54	0.29	0.25
20	7.00 to 7.25	Supper					
20	8.30 to 8.45	29	0.153	513.0	0.49	0.25	0.24
20	10.40 to 10.55	28	0.163	459.6	0.55	0.38	0.17

Here, as in the preceding experiments, there is noticeable the same diminution of amylolytic power, alkalinity, and content of solid matter, etc., in the mixed saliva secreted directly after the morning meal. Of special significance, however, is the marked variation in the values throughout the day, thereby suggesting the existence of a normal curve of secretion. Thus, after the morning meal the saliva shows the effect of the stimulation by its lower content of solids etc. Soon after, however, there is an upward tendency; the curve rises, and amylolytic power is increased as well as the alkalinity, together with the total solids and organic matter. The inorganic salts, on the other hand, still remain low. Towards noon time, amylolytic power sinks very greatly, and there is a corresponding drop in the proportion of organic solids, although the alkalinity and inorganic salts still remain fairly high. After this, amylolytic power gradually rises, reaching the maximum again at 5 P. M. with a corresponding rise in alkalinity, total solids, etc. Supper at 7 P. M. apparently causes a slight fall in amylolytic power, together with a fall

in the solid matter secreted.   At 10.40 P. M. amylolytic power
shows a still greater fall, although alkalinity and solid matter
are increased in amount.

How far are the preceding variations in the secreted saliva
due to the combined influence of taking food and the mechani-
cal stimulation incidental to mastication of the rubber, and
how far to a natural variation in the composition of the
secretion ? This question we have endeavored to answer by
noting the variations in the saliva on a day when food was
abstained from, and by collecting the saliva without move-
ment of the jaws.   This was accomplished by simply resting
the head on the hands, with the mouth downwards, and
allowing the saliva to drip into a beaker without any unneces-
sary movement.*   In this way 15–20 c. c. of saliva were
collected in half an hour.

Following are the results obtained : —

Date.	Time.	Volume Saliva.	Alkalinity Calculated as Na_2CO_3	Amylolytic Power. Milligrams Maltose.	Total Solids.	Organic Matter.	In-organic Salts.
		c.c.	per cent.		per cent.	per cent	per cent.
Jan. 26	Midnight. 11.45 to 12.15	15	0.081	490.4	0.38	0.23	0.15
27	A.M. 6.40 to 7.30	13	0.088	572.4	0.63 †	0.47	0.16
27	9.30 to 10.00	15	0.092	558.6	. . .	. . .	. . .
27	11.00 to 11.30	20	0.071	381.0	0.33	0.18	0.15
27	P.M. 12.25 to 12.50	17	0.102	441.0	0.37	0.19	0.18
27	2.15 to 2.45	16	0.092	347.4	0.32	0.20	0.12
27	4.00 to 4.30	19	0.091	416.4	0.35	0.19	0.16
27	5.15 to 5.50	18	0.102	423.0	0.35	0.19	0.16
27	7.00 to 7.25, Supper						
27	8.30 to 9.00	20	0.102	403.2	0.43	0.28	0.15

A study of these results shows clearly that when the stimu-
lating influences of food and mastication are withdrawn, con-
spicuous alterations in the composition and physiological action
of the saliva are still found, as though there might be a normal

* See Hofbauer, loc. cit., p. 508.
† This result is of somewhat questionable accuracy, having been obtained
with a very small amount of saliva.

curve independent of the fluctuations induced by stimuli. Thus at 11.30 A. M. there is seen the same fall in amylolytic power, that was so conspicuous in the preceding experiment. Further, the saliva secreted at 2.15 P. M. shows a diminution in amylolytic power, as noticeable as the diminution frequently observed after a hearty meal. It is thus quite evident that in the absence of food and other stimulation hourly changes in the amylolytic power of mixed saliva may occur just as marked as those noticed in the saliva secreted before and after breakfast. Variations in alkalinity, total solids, etc., are not so prominent. It is to be noticed, however, from the last series of experiments, that in the absence of breakfast there is no great variation in the amylolytic power of the saliva secreted between 6.40 and 11.00 A. M.; consequently we may accept the conclusion, justified by the results of most of our experiments, that the taking of food, as at breakfast, tends to lower the starch-digesting power of the saliva secreted some time thereafter. This being so, it seems probable that other forms of stimulation may likewise give rise to a change in the composition and physiological action of mixed saliva.

V. Influence of Various Stimuli on the Composition and Amylolytic Power of Human Saliva.

In this series of experiments the attempt was made to ascertain how far the character of the stimulus modifies the properties of mixed saliva. The special stimuli employed were ether, alcohol, whiskey, and gin. The first two were taken into the mouth in the form of vapor, and the saliva allowed to trickle from the mouth without motion of the jaws, the fluid so obtained being compared with saliva resulting from the mechanical stimulation produced by chewing a piece of rubber. With whiskey and gin, the mouth was well rinsed with the fluid and the saliva collected by allowing it to flow from the corner of the mouth. The control experiments with water were made in the same way; *i. e.*, the mouth was rinsed with water and the saliva allowed to trickle forth. Finally, for the sake of comparison and to ascertain how far two samples of

saliva obtained at such close intervals, under similar forms of stimulation, differ from each other, four control experiments were tried with water and rubber alone.

Following are the results obtained: —

Date.	Time.	Stimulus.	Volume Saliva.	Alkalinity calculated as Na_2CO_3.	Amylolytic Power. Milligrams Maltose.	Total Solids.	Organic Matter.	Inorganic Salts.
	A.M.		c.c.	per cent.		per cent.	per cent.	per cent.
Dec. 3	11.05–11.30	Rubber.	40	0.168	582.6	0.63	0.31	0.32
	11.30–11.50	Ether.	30	0.204	624.6	0.76	0.54	0.22
9	9.50–10.10	Rubber.	30	. . .	562.8	0.54	0.30	0.24
	10.10–10.30	Ether.	25	. . .	498.6	0.54	0.29	0.31
13	11.40–12.00	Rubber.	40	0.122	472.2	0.41	0.21	0.20
P.M.	12.00–12.35	Alcohol.	28	0.132	510.6	0.43	0.19	0.24
14	A.M. 10.00–10.30	Water.	30	0.061	473.4	0.32	0.19	0.13
	10.30–11.00	Whiskey.	35	0.102	485.4	0.42	0.29	0.13
16	10.15–10.40	Water.	23	0.071	483.6	0.34	0.20	0.14
	10.45–11.20	Gin.	24	0.102	642.0	0.53	0.36	0.17
17	10.20–10.38	Ether.	27	0.122	586.2	0.32	0.16	0.16
	10.45–10.55	Rubber.	28	0.183	577.2	0.52	0.24	0.28
20	11.15–11.48	Water.	24	0.071	606.6	0.68	0.55	0.13
P.M.	12.15–12.45	Water.	24	0.102	564.0	0.38	0.27	0.11
Jan. 11	3.03– 8.35	Water.	26	0.053	436.8	0.30	0.16	0.14
	4.05– 4.40	Water.	30	0.081	532.2	0.35	0.21	0.14
13	A.M. 11.25–11.40	Rubber.	30	0.153	571.8	0.49	0.26	0.23
P.M.	12.10–12.26	Rubber.	30	0.261	550.8	0.47	0.24	0.23
14	A.M. 10.38–10.58	Rubber.	34	0.132	577.8	0.50	0.27	0.23
	11.30–11.45	Rubber.	32	0.142	594.6	0.51	0.26	0.25

A glance through these results shows at once certain marked differences in the character of the saliva obtained under the various conditions specified. Thus, saliva which flows from the mouth after the latter has been rinsed once with water invariably shows a lower degree of alkalinity, and generally contains a smaller percentage of solid matter than the secretion obtained by the other methods. In amylolytic power, however, there is great variation, some samples showing a relatively strong amylolytic action, while others with essentially the same degree of alkalinity are much weaker in their starch-

digesting power. Simple mastication of rubber has a marked influence in raising the content of alkaline salts in the saliva, as well as the total inorganic constituents, and there is a tendency toward increase in amylolytic power, although the latter is not constant.

As to the influence of alcohol, ether, gin, and whiskey, there is, we think, no question that these agents taken into the mouth change the character of the secretion, increasing its alkalinity, amylolytic power, and content of solid matter. This is certainly true if the secretion so obtained is compared with the saliva flowing from the mouth without stimulation of any kind. Saliva, however, secreted under the stimulation produced by chewing rubber is, as we have seen, comparatively concentrated, and the difference between the secretion resulting from that method and the fluid coming from ether, alcohol, and other like forms of excitation, without mechanical stimulation, is not so decisive in the above experiments as to make the matter quite clear, especially in view of the fact that two portions of saliva obtained one after the other, by the same method of stimulation, are liable to show marked differences in composition and reaction. Particularly noteworthy is the fact that of two portions of saliva collected one after the other by mechanical stimulation (chewing rubber) or by simply allowing the saliva to flow from the mouth after once rinsing the latter with water, the latter portion of saliva is, as a rule, more concentrated and possessed of higher amylolytic power than the portion first secreted. It is thus obvious that great care must be exercised in drawing deductions from the composition and amylolytic action of mixed saliva when the latter is so prone to vary under what seem to be essentially the same forms of stimulation. It is furthermore equally obvious that the possible causes to which the above variations may be attributed are many, since there are involved three distinct sets of glands in addition to the buccal glands of the mouth cavity. Hence, increase or decrease in amylolytic power, as well as in the general concentration of the secretion, may involve simply an alteration in the relative activity of the individual glands and

not be connected primarily with any specific stimulation of metabolic or secretory activity.

However this may be, it is quite clear that the natural variations in the character of the mixed saliva, indicated by the results of the last four experiments of the above series, render it necessary to use great caution in arranging the conditions under which the experiments are tried. We have therefore repeated the above experiments, choosing for the collection of the saliva a time of day when we have found the mixed saliva most constant in composition, viz., between 9.30 and 10.30 A. M. To be sure, there are variations in the composition and starch-digesting power of successive portions of saliva collected by the same method at this period, but they are relatively small, — quite small, indeed, as compared with the variations liable to occur at other periods of the day. The truth of this statement is illustrated by the two following experiments, in which the saliva was collected without stimulation, simply allowing it to flow from the mouth.

Date.	Time.	Volume Saliva.	Alkalinity as Na_2CO_3.	Amylolytic Power. Milligrams Maltose.	Total Solids.	Organic Constituents.	Inorganic Salts.
	A.M.	c.c.	per cent.		per cent.	per cent.	per cent.
Feb. 3	9.32 to 10.06	21.0	0.0816	569.4	0.50	0.31	0.19
3	10.15 to 10.42	22.0	0.0918	549.0	0.46	0.29	0.17
	P.M.						
3	5.00 to 5.20	19.5	0.0918	573.6	0.49	0.31	0.18
3	5.27 to 5.50	17.0	0.1122	613.8	0.68	0.51	0.17

Thus, the two portions collected between 9.32 and 10.42 A.M. are essentially alike, while the two fractions secreted between 5.00 and 5.50 P.M., all without stimulation, are more dissimilar. Adopting the morning hour as the better time for collection, experiments were tried with alcohol, ether, chloroform, whiskey, and gin, comparing in each case the saliva obtained under their influence with the secretion coming without stimulation of any kind. The exact method pursued in the case of the control, *i. e.,* with water, was to rinse the

mouth once with distilled water, after which the saliva was simply allowed to drop from the mouth into a beaker. With ether and chloroform the mouth was filled once with the vapor and the saliva then allowed to flow spontaneously into a receptacle without any motion of the jaws. With the alcohol, gin, and whiskey, 10 c.c. of the fluid were taken into the mouth, held a moment, and then ejected, after which the saliva was collected as in the other cases. Lastly, an experiment was tried (Feb. 15) by chewing rubber as a stimulant, and comparing the saliva so obtained with a control secreted without stimulation. Following are the results obtained:

Date.	Time.	Stimulus.	Vol. Saliva.	Alkalinity as Na_2Co_3.	Amylolytic Power. Milligrams Maltose.	Total Solids.	Organic Constituents.	Inorganic Salts.
			c.c.	per cent.		per cent.	per cent.	per cent.
Feb. 7	A.M. 10.05–10.32	Water.	18.0	0.0714	480.6	0.42	0.22	0.20
	10.37–10.56	40% alcohol.	18.0	0.1122	514.2	0.43	0.26	0.17
8	9.37–10.05	Water.	18.0	0.0612	566.4	0.42	0.25	0.17
	10.11–10.32	Ether.	18.0	0.1122	558.6	0.54	0.29	0.25
10	9.53–10.18	Water.	17.5	0.0816	604.2	0.51	0.33	0.18
	10.27–10.47	Chloroform.	17.0	0.0714	644.4	0.69	0.48	0.21
11	9.40–10.07	Water.	17.0	0.0714	493.3	0.39	0.25	0.14
	10.14–10.36	Whiskey.	17.0	0.1020	547.8	0.50	0.31	0.19
15	9.52–10.16	Water.	16.5	0.0816	541.2	0.38	0.21	0.17
	10.21–10.27	Rubber.	17.0	0.1530	577.2	0.58	0.26	0.32
18	9.33–10.03	Water.	17.0	0.0714	584.4	0.49	0.33	0.16
	10.10–10.34	Gin.	19.0	0.1020	610.2	0.57	0.39	0.18
23	9.26– 9.51	Water.	17.0	0.0714	429.6	0.30	0.18	0.12
	10.01–10.24	Water.	17.5	0.0714	423.0	0.31	0.18	0.13

From these results it would seem quite clear that the several agents employed, with the exception of chloroform, give rise to a marked increase in the content of alkaline-reacting salts in mixed saliva. Mechanical stimulation, as by chewing rubber, however, is even more effective than the chemical stimuli employed, although it must not be overlooked that in the above experiments the action of alcohol, ether, whiskey, etc., is necessarily of short duration. Further, there is evidence in most of the results of an increase in amylolytic power, as well

as in the content of solid matter under the influence of the stimuli. It is thus safe to assert that alcohol and alcoholic fluids not only stimulate the flow of saliva, but that they also tend to increase the concentration and amylolytic power of human mixed saliva, — results which are in close accord with the action of these fluids upon the secretion of the sub-maxillary saliva of the dog.* Further, simple mechanical stimulation, as mastication, may also increase the amylolytic power of mixed saliva. Lastly, it should be mentioned that the saliva resulting from the above forms of stimulation, excepting mechanical stimulation, is much more viscid than the fluid secreted spontaneously, evidently from a higher content of mucin.

Summary.

Human mixed saliva contains normally no sodium carbonate whatever; the alkalinity indicated by litmus, lacmoid, etc., is due to hydrogen alkali phosphates, with possibly some alkali bicarbonate. Mixed saliva invariably reacts acid to phenolphthaleïn.

The alkalinity of mixed saliva, as indicated by lacmoid, is greater before breakfast than after the morning meal; a conclusion which stands in direct opposition to the statement frequently made that "the alkalinity (of mixed saliva) is least when fasting, as in the morning before breakfast, and reaches its maximum with the height of secretion during or immediately after eating." †

Saliva secreted after a period of glandular inactivity, as before breakfast, manifests greater amylolytic power than the secretion obtained after eating, as observed by Hofbauer. Corresponding with this increase in amylolytic power occurs an increase in the proportion of alkaline-reacting salts, but the increased amylolysis is due primarily to an increase in the amount of active enzyme contained in the saliva.

Mixed saliva, whether collected by mechanical stimulation

* See Chittenden, Mendel, and Jackson, Amer. Jour. Physiol., 1898, i, p. 167.
† Text-book of Physiology, edited by E. A. Schäfer, 1898, i, p. 344.

or collected without effort, shows a natural tendency to vary both in composition and in amylolytic power throughout the twenty-four hours, and apparently independent of the taking of food. Between 7.00 and 11.00 A.M., however, in the absence of food the secretion is remarkably constant.

Mechanical stimulation, as chewing a tasteless substance, and alcohol, ether, gin, whiskey, etc., taken into the mouth, all lead to the outpouring of a secretion richer in alkaline-reacting salts and in amylolytic power than the secretion coming without stimulation.

Mixed saliva resulting from stimulation with ether, alcohol, etc., contains a much larger proportion of mucin than the secretion coming without stimulation, being noticeably thick and viscid. This quality is not apparent in the saliva resulting from mechanical stimulation.

ON THE PATHS OF ABSORPTION FOR PROTEIDS.[*]

By LAFAYETTE B. MENDEL.

THE text-books of physiology agree, at present, in assigning
to the portal circulation the task of transporting away from
the alimentary canal ingested proteids after they have been
modified by the natural digestive processes. The experimental
evidence in favor of this view is familiar, and has scarcely been
questioned; recently, however, Asher and Barbèra [†] have
published the results of an experiment which leads them to
conclude that the thoracic duct forms — though perhaps only
to a small extent — a channel for the transportation of digested
proteids to the blood. These investigators experimented upon
a large dog having a well-healed gastric fistula. A cannula
was introduced into the thoracic duct of the animal after it
had fasted for sixty hours. During continued narcosis the
hunger-lymph was collected for an hour; two hundred grams
of dry albumin (from blood) were then introduced into the
stomach through the fistula and thereupon the lymph collected
for six hours in hourly portions. Total solids, ash, and total
nitrogen were determined in the seven portions, and the pro-
teid content of the lymph was calculated from these data.
The results obtained may be summarized as follows:

Experiment of Asher and Barbèra.

AVERAGE COMPOSITION OF LYMPH, PER HOUR.

		Before Proteid Feeding.	After Proteid Feeding.
Lymph,	grams	16.50	27.91
Total solids	grams	1.15	2.18
" "	per cent	7.05	7.81
Ash	grams	0.08	0.10
"	per cent	0.49	0.35
Nitrogen	grams	0.132	0.251
"	per cent	0.809	0.899
Proteid (N × 6.25)	grams	0.825	1.572
" "	per cent	5.056	5.632

[*] Reprinted from the Amer. Jour. of Physiol., vol. ii.
[†] Asher and Barbèra: Centralblatt f. Physiologie, 1897, xi, p. 403. "Der

In connection with the data obtained, Asher and Barbèra point out that the curves plotted to indicate the changes in the composition of the lymph from hour to hour show a striking similarity to those obtained for the hourly nitrogen excretion in the urine after meals;[*] and the authors are inclined to attach significance to the correspondence between the curves.

In support of earlier experiments[†] demonstrating that — in man — the absorption of ingested proteids is not ordinarily a function of the lymphatics, I. Munk[‡] has published a brief review of the experiment of Asher and Barbèra. He points out that the total proteid found in the lymph during the six hours of the absorption period exceeds the proteid in the hunger-lymph, calculated for an equal period, by only 4.485 grams, *i. e.*,

Total proteid in " absorption " lymph   = 9.435 grams
     "   "   " hunger "   " (6×0.825) = 4.950   "
Proteid presumably absorbed into lymphatics   = 4.485   "

Of the 200 grams of proteid introduced into the dog's stomach, 130 grams were recovered at the end of the experiment. Accordingly, since 70 grams of the albumin introduced had disappeared from the alimentary tract, the additional quantity of proteid found in the lymph during six hours of absorption corresponds to only 6.4 per cent of the proteid absorbed. In other words, 93.6 per cent of the material absorbed presumably passed into the circulation by channels other than those leading to the thoracic duct. Munk further points out that these results were obtained with excessive quantities of proteid : 200 grams being given to the dog at one time.

Brustgang betheiligt sich also nach Eiweissnahrung an der Fortführung des Eiweisses in das Blut, wenn auch in geringer Menge." Ibid., pp. 406–7.

   * Cf. Tschlenoff, Correspondenzblatt f. Schweizer Aerzte, 1896, p. 1; Veraguth, Journal of Physiology, 1897, xxi, p. 112; Hopkins and Hope, ibid., 1898, xxiii, p. 271; Hopkins, Schaefer's Text-book of Physiology, 1898, i, p. 585.

   † Munk and Rosenstein, Virchow's Arch. f. d. exper. Pathologie, 1891, cxxiii, p. 496.

   ‡ I. Munk, Centralblatt für Physiologie, 1897, xi, p. 585.

I have repeated the experiment of Asher and Barbèra with modifications in two respects. First, a smaller quantity of proteid was fed; and secondly, the proteid employed was a readily soluble one — Witte's "pepton" — which previous experience had shown to be satisfactorily utilized by dogs.[*] A gastric fistula had been made on the 14-kilo animal several months previously. The wound was perfectly healed and the dog in good health.[†] After forty-eight hours' fasting a glass cannula was tied into the thoracic duct during morphine narcosis (0.16 gram morphine sulphate + 0.016 gram atropine sulphate subcutaneously), a little chloroform-ether being administered when necessary. The dog remained in narcosis during the experiment. The portion of lymph collected during the first ten minutes after introduction of the cannula was not retained, since owing to the operative procedure the flow of lymph is at first always somewhat more rapid than subsequently.[‡] The "hunger" lymph was collected during an hour; thereupon 55 grams of Witte's "pepton" containing 14.0 per cent nitrogen were introduced with 125 c.c. water through the fistula into the stomach. The lymph was then collected in hourly portions during the six succeeding hours. The animal was killed by bleeding and the stomach contents removed and examined. The intestine was found empty; the appearance of the gastric and intestinal mucosa was normal. The stomach contained 205 c.c. fluid with mucous flocks in suspension. The filtered gastric contents, composed largely of primary proteoses, with a little true peptone, furnished the following data on analysis:

Total solids	30.23	grams
Total nitrogen (Kjeldahl)	4.42	"
Nitrogen in total solids	14.60	per cent
Total acidity (equivalent to HCl)	0.77	"

No *free* HCl present.

[*] Cf. Mendel and Jackson, Amer. Jour. Physiol., 1898, ii, pp. 10–11.

[†] The form of gastric cannula used has been described in the American Journal of Physiology, 1898, i, p. 191.

[‡] Cf. Heidenhain, Arch. f. d. ges. Physiol., 1891, xlix, p. 215.

The lymph portions were analyzed in the customary manner, nitrogen being determined by the Kjeldahl method and the proteid calculated from the figures thus obtained. The results are summarized in the accompanying table.

TABLE OF ANALYSES OF LYMPH.

Time.	Amount Collected.	Total Solids.		Ash.		Nitrogen.		Proteid (N × 6.25).	
hours.	grm.	grm.	per cent.	grm.	per cent.	grm.	per cent.	grm.	per cent.
1. (Before feeding).	13.46	0.551	4.09	0.122	0.90	0.0609	0.45	0.381	2.83
2. (After feeding).	16.59	0.625	3.83	0.147	0.88	0.0674	0.40	0.421	2.53
3. " "	11.50	0.472	4.10	0.104	0.90	0.0475	0.41	0.297	2.58
4. " "	12.64	0.548	4.33	0.105	0.83	0.0614	0.48	0.384	3.03
5. " "	8.79	0.393	4.47	0.068	0.77	0.0454	0.51	0.284	3.23
6. " "	14.60	0.674	4.61	0.125	0.85	0.0768	0.52	0.480	3.28
7. " "	15.90	0.708	4.45	0.128	0.80	0.0757	0.47	0.473	2.97
Six fasting hrs. (calculated).	80.76	3.306	4.09	0.732	0.90	0.3654	0.45	2.286	2.83
Six absorption hrs. (2 — 7).	80.02	3.420	4.27	0.677	0.84	0.3742	0.46	2.339	2.92

The 55 grams of Witte's "pepton" introduced into the stomach contained 7.70 grams of nitrogen. At the end of the experiment unabsorbed proteid containing 4.42 grams nitrogen was recovered from the stomach. The nitrogen in the "pepton" absorbed thus amounted to 7.70 — 4.42 = 3.28 grams, equivalent to 23.4 grams of Witte's "pepton." Nearly one-half the proteid fed had thus been absorbed; in Asher and Barbèra's experiment about one-third of the albumin disappeared. In my experiment the total nitrogen in the lymph during the absorption period amounted to 0.374 gram; the nitrogen-content of the starvation lymph (1) calculated for an equal period, would amount to 6 × 0.0609 = 0.365 gram. The difference between these figures — 0.0088 gram N = 0.053 gram proteid — which may be attributed to the proteid absorbed, is insignificantly small in amount, and is far smaller than the corresponding figure obtained by Asher and Barbèra with a considerable larger quantity of proteid. Neither the total quantity of lymph collected nor the percentage of proteid present was increased during the six hours of absorption.

With reference to a possible correspondence between lymph flow and urea excretion after proteid absorption it may be remarked that — according to Tschlenoff — if peptone is ingested instead of ordinary proteids, the maximum urea excretion is reached in the second hour.   To emphasize the comparison — as Asher and Barbèra have done — between the lymph flow and the urea excretion observed in quite different experiments seems to me of doubtful value; for the lymph flow is readily influenced by purely mechanical factors.   Thus it is quite possible that, in my experiment, movements of defecation observed in the dog in the sixth hour may explain the increased flow of lymph unaccompanied by any alteration in the percentage of proteids; just as slight pressure on the abdomen will immediately cause an increase in the flow.

Asher and Barbèra [*] have formulated the theory that the lymph is a product of metabolism in the glands of the body, and they have attempted to demonstrate that the lymph flow increases in all cases where there is accelerated glandular activity.   In view of the work accomplished by the alimentary epithelium and the liver during the digestion and absorption of proteids, the new theory demands that an increased flow of lymph should result after proteid feeding.   The present experiment with " pepton " failed to yield results in conformity with this view; it might, however, be said by the defenders of the theory that no pronounced increase in lymph flow is to be expected during the absorption of soluble and readily diffusible products like Witte's " pepton," since the work of the intestines is comparatively slight under these conditions.   Such, at least, is the explanation which they have applied to the absence of accelerated lymph flow in carbohydrate nutrition.

The results of the absorption experiment with moderate quantities of a soluble proteid fail to modify the current statements regarding the paths by which proteids are absorbed; *i. e.*, under ordinary circumstances by far the greater share in the process must still be delegated to the capillaries of the villi.

---

[*] Asher and Barbèra: Zeitschr. f. Biologie, 1898, xxxvi, p. 210; Asher, IV. International Physiological Congress, Centralbl. f. Physiol., 1898, xii, p. 486.

A CHEMICO-PHYSIOLOGICAL STUDY OF CER-
TAIN DERIVATIVES OF THE PROTEIDS.[*]

By R. H. CHITTENDEN, LAFAYETTE B. MENDEL, AND YAN-
DELL HENDERSON.

THE marked activity which has characterized the study of
the proteids and their primary digestion or cleavage products
during the last decade has been especially manifest in two dis-
tinct directions. In the first place, effort has been directed
toward a study of the various transitions which these substan-
ces undergo in the several phases of digestion and nutrition,
with a view to obtaining clearer insight into the genetic rela-
tionship of the various chemical products and incidentally into
their chemical nature. In the second place, much attention
has been given to a study of the physiological action of the
more characteristic products of gastric and pancreatic proteoly-
sis, and more especially the behavior of these products when
introduced into the system through channels other than the
gastro-intestinal tract. This latter phase of physiological
research has been carried out in the majority of cases with a
mixed product resulting from the digestion of blood fibrin
with gastric juice, known as "Witte's pepton," and composed
of a variable proportion of proteoses with some true peptone.

It is needless to refer here in detail to the extensive litera-
ture which these latter investigations have created. It will
suffice to recall the fact that the more important observations
on the effect of so-called "peptones" when introduced directly
into the blood current were made by Schmidt-Mülheim [†] and
Fano [‡] in Ludwig's laboratory. The results they obtained
have in part become classic, and supplemented by certain later
observations made by other investigators may be briefly sum-

[†] Schmidt-Mülheim, Archiv f. Physiol., 1880, p. 30.
[‡] Fano, Ibid., 1881, p. 277.

marized as follows: The primary products of digestion (proteoses), when introduced directly into the circulation of dogs in doses from three to five decigrams per kilogram of body-weight, produce a rapid and marked fall of arterial blood-pressure, which slowly returns to the normal. If after the injection of the proteoses blood be withdrawn from an artery, it fails to clot as rapidly as under normal conditions, or may even remain liquid indefinitely. The intensity of the effects produced depends in part on the rapidity with which the substance is introduced into the circulation and to a lesser degree on the quantity of material employed. Further, when the effects of an injection have disappeared, a second similar injection may fail to produce the characteristic action, a certain degree of immunity having been established. Coincident with these results there usually occurs a very marked increase in the flow of lymph in the thoracic duct, together with a decreased coagulability of this fluid. Other changes in the urinary secretion and respiration are less constant, and have been less thoroughly investigated.

These statements will, we believe, receive general acceptance, as expressing well-established truths. There remain, however, a large number of problems to the solution of some of which the experimental work of this paper is directed. Thus the questions arise: Are the various physiological results — fall of arterial pressure, loss of blood coagulability, lymphagogic influence, etc. — genetically related or independent phenomena? Is the influence exercised directly upon the blood or indirectly through specific organs or tissues? Are all organisms equally susceptible to these actions? Are the effects produced common to all decomposition or cleavage products of proteids? What light do the phenomena throw upon normal or abnormal processes in the body?

Such are some of the problems presented. Recent work by a number of investigators, however, has thrown light in many directions and revealed a multitude of facts which promise advance in this particular field. Still, few investigators have attempted to use for study more characteristic or more definite

chemical products than the earlier workers had at their command, or where this has been attempted there has in some cases at least existed a certain degree of indefiniteness concerning the exact nature of the substances employed. This is the more to be regretted as there are many facts which tend to show that the primary cleavage products of the proteids may differ from each other in their chemical constitution, and if so one might naturally look for pronounced variations in physiological behavior. Further, it unfortunately happens that previous experimental data obtained with isolated proteoses and peptones are frequently deficient or entirely wanting on many points of interest. We therefore present our results obtained with various types of proteid cleavage products in the hope that they may contribute somewhat to a broader and more concise knowledge of the subject. The several products we have made use of are fully described as regards mode of preparation, composition, and chemical characters in another section of this paper. Their physiological action is described in the following pages.

THE PHYSIOLOGICAL EFFECTS OF SOME CLEAVAGE PRODUCTS OF THE PROTEIDS WHEN INTRODUCED DIRECTLY INTO THE CIRCULATION.

Technique. — Our experiments were carried out exclusively on dogs. The animals were made to fast for at least twenty-four hours previous to the operative procedure; they were narcotized by a subcutaneous injection of morphine mixed with atropine, and a mixture of chloroform and ether was administered during the operative interference and at other times when necessary. Arterial pressure was recorded by a mercurial manometer connected with the carotid or femoral artery, and the injections were made into one of the facial veins from a burette attached to a glass cannula. Intervals of one second were recorded beside the blood pressure curve by means of a metronome time-marker. In observing the rate at which the blood coagulated, portions were drawn from the right femoral artery. The cannula was removed after each withdrawal of

blood and thoroughly cleansed, and the blood was allowed to escape for a second or two before the next portion was taken. The samples of 2 c.c. each were received in graduated cylinders with a caliber of one centimeter. The "rate of coagulation" was taken to be the time which elapsed before the cylinder could be inverted without the loss of a drop.

The elimination of the substances through the kidneys, as well as the rate of flow of the urine, was studied by collecting the urine from cannulæ introduced directly into the ureters. In those experiments in which the lymph was collected and analyzed, the directions of Heidenhain * were followed.

The proteoses, peptones, etc., studied were usually dissolved in 0.7 per cent sodium chloride solution. With substances insoluble in dilute saline fluids, such as heteroalbumose and antialbumid, it was found best to use a few drops of ammonium hydroxide to facilitate solution, any excess of ammonia being driven off by heat. The volume of fluid injected was ordinarily 4 c.c. per kilo of body-weight up to a maximum of 50 c.c. The doses of the different substances employed were varied in order to ascertain their relative activity. The injections were accomplished as rapidly as possible, and seldom occupied more than thirty seconds. Indeed, many of the tracings show a slight initial rise of blood-pressure due probably to the increased volume of the blood reaching the heart, before the fall caused by the substances injected.

Specific substances studied. — In selecting substances for study we have been influenced by the fact that the evidence already at hand is sufficient for establishing the general action of mixed proteoses and peptones. It has therefore seemed to us desirable to study especially the physiological action of certain well-defined cleavage products with a view to obtaining evidence of the action of the individual bodies present in the ordinary digestion products of the proteids. Thus, an investigation of the physiological action of the immediate precursors of antipeptone has seemed especially desirable in view of the discordant observations of previous investigators with this

* Heidenhain, Archiv f. d. ges. Physiol., 1891, xlix, p. 209.

product and also in view of the peculiar significance which is attached to this substance through recent chemico-physiological research.[*] Furthermore, we have been impressed with the importance of having true antipeptone for experiment, and consequently, we have employed pure antialbumid as the mother substance from which to prepare the antipeptone. Following is a list of the substances studied, all of which were prepared with great care according to the methods detailed in the second part of this paper.

Antialbumid (A), formed from coagulated egg-albumin by the action of dilute sulphuric acid at 100° C., and purified by digestion with pepsin-acid, etc.

Antialbumoses (B), formed by the digestion of the above antialbumid with gastric juice.

Antialbumoses (C), formed by the digestion of the above antialbumid with pancreatic juice.

Antipeptone (D), formed by the digestion of antialbumid with pancreatic juice.

Antialbumid (E), a more insoluble form, separating from an alkaline solution of the above antialbumid when subjected to pancreatic digestion.

Antialbumid (F), prepared from crystallized edestin by the action of dilute sulphuric acid.

Hemialbumoses (G), formed from coagulated egg-albumin by dilute sulphuric acid.

Heteroalbumose (H), formed in a similar manner.

Hemipeptone (I), formed by the same method as the hemialbumoses.

Protogelatose (J), formed from pure gelatin by gastric digestion.

Deuterogelatose (K), formed by pancreatic digestion.

Gelatin-peptone (L), formed by pancreatic digestion.

Albumose-like body (M), formed from coagulated egg-albumin by the action of dilute sulphuric acid at 100° C.

Influence on blood-pressure. — The first more comprehensive and systematic study of the physiological action of proteoses and peptone individually was made by Grosjean.[†]   This

---

[*] Siegfried et al.

[†] Grosjean, Archives de biologie, 1892, xii. This paper contains references to the earlier literature.

investigator prepared the several products from blood-fibrin by artificial gastric digestion, using the methods of Kühne and Chittenden in isolating and purifying the mixed proteoses and peptone. Two products only were prepared, *i. e.*, "propeptone," a varying mixture of primary and secondary proteoses, and "peptone" or amphopeptone. These products were introduced intravenously into the circulation of dogs and other animals, and arterial blood-pressure registered. Grosjean concluded from his experiments that "propeptone" introduced into the circulation causes a considerable fall of arterial pressure, the depression occurring at once after the injection and quickly reaching its maximum. Further, the extent to which arterial pressure is lowered is apparently not influenced by the size of the dose, except when the latter amounts to less than 15 centigrams per kilo of body-weight, in which case the depression is much less marked. Moreover, as a rule, the pressure once lowered is very slow in returning to the normal. Thus, in one experiment where 1 gram of "propeptone" per kilo was injected, arterial pressure was still below the normal three and a half hours after the injection. With peptone, there is likewise produced a fall of arterial pressure which, however, is less marked, slower in reaching its maximum, and of shorter duration than that caused by like doses of "propeptone." Further, when the pressure returns toward the normal it frequently rises slightly above the original height, after which it again falls, although this second depression is slight as compared with the first.

Arthus and Huber [*] working with gelatoses and caseoses formed by pancreatic digestion, observed that when these bodies were introduced intravenously into dogs in large doses (2 grams gelatoses per kilo; 1.5 grams caseoses per kilo), and the injections carried out rapidly, there was a marked lowering of blood-pressure comparable to that obtained with the fibrin proteoses. Here, too, no attempt was made to differentiate the mixed caseoses or gelatoses into more specific products.

* Arthus and Huber, Archives de physiologie, 1896, p. 857.

In a recent paper from this laboratory * attention was called to the action of deuteroalbumose and peptone obtained by the proteolysis of egg-albumin with papaïn. The experiments showed an immediate lowering of blood-pressure when these products, in doses of 0.5 gram per kilo of body-weight, were injected into the circulation, the pressure, however, soon returning to the normal.

Thompson † has investigated the specific action of Witte's "pepton" on the vasomotor system, and more recently this study has been extended ‡ to include the action of pure peptone, antipeptone, and deuteroalbumose, although the details of these latter experiments have not yet been published. The results obtained agree in a general way with those already recorded by Grosjean. They indicate, however, that smaller doses of these products than has generally been supposed, suffice to produce physiological effects. It is furthermore definitely shown that lowering of arterial pressure is due to a vascular dilatation as the result of a peripheral or local action on the blood-vessels. No central nervous influences could be demonstrated. This vasomotor action is not confined to the splanchnic area, but is common to all the blood-vessels, and is exercised presumably upon the endings of the vasomotor nerves and on the muscles of the blood-vessels, — the decreased irritability of the neuro-muscular apparatus making the latter incapable of responding to the normal vaso-constrictor impulses. In addition, it is to be noted that Thompson observed that deuteroproteose produced a far more profound and enduring influence on arterial pressure than pure peptone, but less so than the same dose of Witte's "pepton." Hence, deuteroproteose cannot be regarded as the most potent constituent of Witte's "pepton," a statement which agrees with Pollitzer's § observations. Lastly, in agreement with other

* Chittenden, Mendel, and McDermott, Amer. Jour. Physiol., 1898, i, p. 255.

† Thompson, Journal of Physiology, 1896, xx, p. 455.

‡ Thompson, Interim report of a committee consisting of Professors Schäfer, Sherrington, Boyce, and Thompson. Report by the Secretary of the British Association for the Advancement of Science, 1896–97.

§ Pollitzer, Journal of Physiology, 1886, vii, p. 283.

workers (Pollitzer, Spiro, and Ellinger [*]) Thompson found that antipeptone contrasts very noticeably with the other products of proteid digestion studied in its action on blood-pressure. The fall of pressure produced was *temporary* at most.

Our experiments on blood-pressure, the results of which are summarized in the following table, indicate that antialbumid, a product whose physiological action has not heretofore been investigated, agrees with the antipeptone derived from it in producing comparatively slight effects on mean arterial pressure. In like doses, however, antialbumid is somewhat more vigorous in action than antipeptone. Further, it is evident that antipeptone derived from antialbumid produces essentially the same results as antipeptone formed by the direct action of pancreatic juice on a natural proteid. Antialbumoses, on the other hand, act most energetically in lowering blood-pressure. In no case was a return of mean arterial pressure to the normal observed within an hour after injection of the various albumoses studied. The same was true of the hemialbumoses. The albumoses of different origin act qualitatively and quantitatively alike in great degree, heteroalbumose being apparently most vigorous in its effects. This corresponds with the observations of Pollitzer,[†] and we are inclined to attribute the pronounced effects produced by Witte's " pepton " to the considerable admixture of heteroproteose, which it has repeatedly been shown to contain.[‡]

Our experiments with hemipeptone agree with the results obtained by Grosjean and later by Thompson with amphopeptone isolated by Kühne's method. Further, between hemipeptone and antipeptone there is little or no difference of action when like amounts of substance are employed. It is interesting, however, to contrast the action of equal doses of hemipeptone (XIX-XX) with that of the hemialbumoses (V) from the same source. It is only with doses of 1.0 gram of hemi-

* Spiro and Ellinger, Zeitschr. f. physiol. Chem., 1897, xxiii, p. 135.
† Pollitzer, loc. cit.
‡ Kühne and Chittenden, Zeitschr. f. Biologie, 1884, xx, p. 15.

TABLE, SHOWING EFFECTS UPON ARTERIAL PRESSURE,
MEASURED IN MILLIMETERS OF MERCURY.

Experiment.	Weight of Animal; kilos.	Grams per kilo Injected.	Nature of the Substance Employed.	Pressure before Injection.	Pressure just after Injection.	5 minutes after.	15 minutes after.	30 minutes after.	1 hour after.	1¼ hours after.	2 hours after.
I.	8.8	0.30	Antialbumoses (B)	180	90	40	45	50	. .	75	. .
II.	6.2	0.30	" "	85	45	30	35	50	70	. .	. .
III.	11.3	0.30	Antialbumoses (C)	140	30	30	80	45	55	95	110
IV.	6.0	0.30	" "	130	35	. .	105	125	. .	. .	. .
V.	18.0	0.30	Hemialbumoses (G)	170	65	40	38	50	65	85	. .
VI.	7.5	0.18	Heteroalbumose (H)	160	135	30	20	30	45	45	45
VII.	9.8	0.20	" "	115	75	30	. .	75	. .	. .	. .
VIII.	7.0	0.30	Antialbumid * (A)	80	20	45	90	90	. .	. .	. .
IX.	6.0	0.30	" "	150	80	40	115	125	. .	. .	. .
X.	5.4	0.30	" "	130	70	125	150	145	150	135	. .
XI.	7.6	0.14	" "	150	55	40	115	125	140	150	. .
XII.	14.0	0.15	" "	140	90	135	130	. .	. .	. .	. .
XIII.	30.0	0.03	" "	65	30	20	45	60	65	. .	. .
XIV.	8.4	0.17	" "	140	140	140	140	140	. .	. .	. .
XV.	6.8	0.07	Antialbumid (F)	95	95	85	110	100	. .	. .	. .
XVI.	9.0	0.30	Antipeptone (D)	170	160	160	190	170	. .	. .	. .
XVII.	6.5	0.30	" "	90	40	80	90	90	. .	. .	. .
XVIII.	9.0	0.30	" "	110	60	135	145	150	. .	. .	. .
XXXI.	4.5	0.45	" "	120	55	70	140	140	. .	. .	. .
XIX.	11.2	0.30	Hemipeptone (I)	125	20	120	105	. .	. .	80	. .
XX.	6.5	0.30	" "	125	60	110	130	130	130	. .	. .
XXI.	13.5	0.50	" "	130	40	85	130	120	145	145	. .
XXII.	6.4	1.00	" "	115	50	20	45	60	110	. .	. .
XXIII.	5.0	0.30	Albumose-like body (M)	110	40	20	50	110	110	. .	. .
XXIV.	5.0	1.50	Protogelatose (J)	140	55	135	150	135	. .	. .	. .
XXV.	4.2	0.30	Gelatin-peptone (L)	100	75	100	95	100	100	. .	. .
XXVI.	6.0	0.60	" "	105	40	60	115	120	115	. .	. .
XXVII.	21.0	0.35	Deuterogelatose (K)	115	115	115	120	120	120	. .	. .
XXVIII.	7.0	0.50	" "	120	50	95	115	120	120	. .	. .
XXIX.	15.0	0.70	" "	130	60	120	150	150	. .	. .	. .
XXX.	6.5	1.00	" "	135	35	65	125	140	120	. .	. .

peptone per kilo that a prolonged fall of pressure is obtainable, and even with this quantity the pressure again resumes its normal within an hour. These facts seemingly suggest more or less pronounced difference in the chemical structure of the proteose and peptone molecules, — a point which we have taken occasion to emphasize in other connections.†

* All of the samples of antialbumid were dissolved by the aid of a little dilute ammonia except in Experiment XIV, where dilute sodium carbonate was used.

† Chittenden and Mendel, Journal of Physiology, 1894, xvii, p. 78. Chit-

The experiments with protogelatose and deuterogelatose in agreement with the observations of Arthus and Huber, fail to show any such marked influence as is obtained with the corresponding albumoses. True gelatin-peptone, on the other hand, approaches hemipeptone and antipeptone in the intensity of its action. The great difference which the albuminoid products show, quantitatively at least, in contrast with the derivatives of the native proteids serves to emphasize the other striking differences between gelatin and true proteids as regards physiological equivalence.*

Lastly, attention is directed to the slight initial rise in pressure immediately succeeding the injection of the various substances into the circulation as shown in the tracings. This rise, which is undoubtedly due to the rapid entrance of the fluid into the vascular system,† gives evidence of the rapidity with which the injections were carried out. This fact is not without significance, since the variation in effects of intravenous injections of proteoses has been demonstrated (cf. Thompson) to be dependent in part on the rapidity of the injections. It seems reasonable, therefore, to assume that the intensity of vasomotor effects is not so much a function directly of the absolute quantity injected as of the quantity in the circulation per unit of time, or, in other words, of the concentration of the substance in the blood.

The accompanying blood-pressure tracings have been selected from typical experiments. They were recorded with a mercurial manometer, as already described. The time is recorded in seconds, and the line of zero pressure is likewise recorded. The curves all read from right to left, and only such portions have been reproduced here as furnish evidence of the observations discussed in the preceding pages.

tenden, Digestive Proteolysis, 1894, p. 42. Also recent papers from Hofmeister's laboratory. Zeitschr. f. physiol. Chem., 1897–98.

* Cf. I. Munk, Archiv f. d. ges. Physiol., 1894, lviii, p. 309. Also Mendel and Jackson, Amer. Jour. Physiol., 1898, vol. ii, p. 21.

† Cf. Thompson, Journal of Physiology, 1896, xx, p. 455.
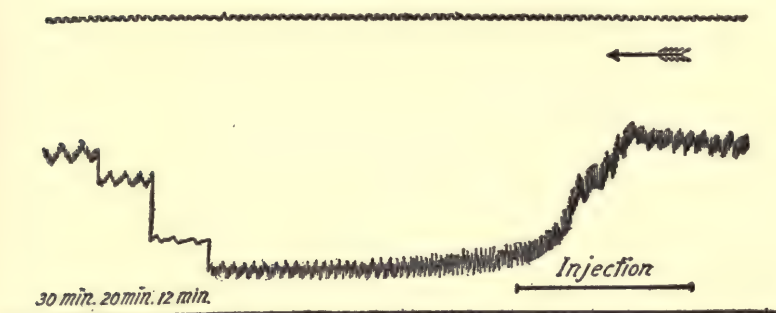
EXPERIMENT IV.   6 kilo dog.   Injection of Antialbumoses (C).   0.3 gram per kilo.
One fourth the original size.
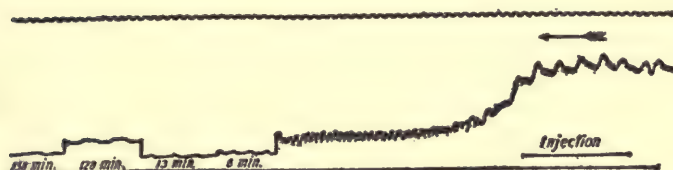
EXPERIMENT VI.   7.5 kilo dog.   Injection of Heteroalbumose (H).   0.18 gram
per kilo.   (Cf. tracing Exper. XXIX *b.*)   One eighth the original size.

EXPERIMENT IX.   6 kilo dog.   Injection of Antialbumid (A).   0.2 gram per
kilo.   One eighth the original size.

19
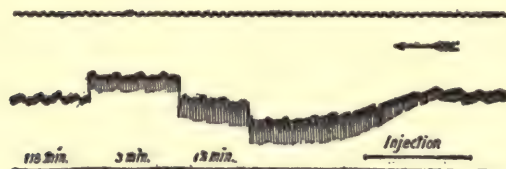
EXPERIMENT XVIII. 9 kilo dog. Injection of Antipeptone (D). 0.3 gram per kilo. One eighth the original size.
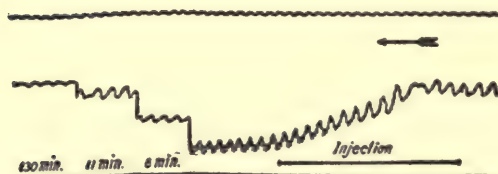
EXPERIMENT XXI. 13.5 kilo dog. Cannula in thoracic duct. Injection of Hemipeptone (I). 0.5 gram per kilo. One eighth the original size.
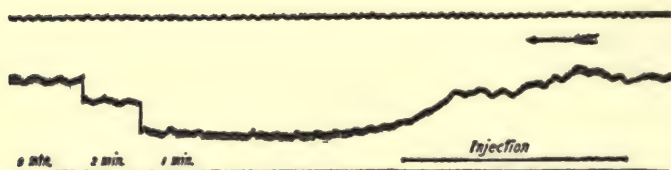
EXPERIMENT XXIV. 5 kilo dog. Injection of Protogelatose (J). 1.5 grams per kilo. One eighth the original size.
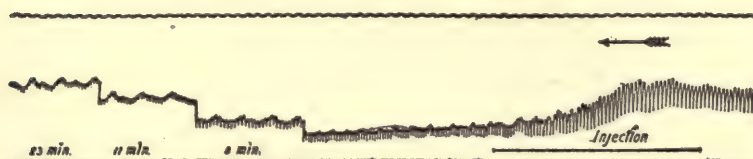
ᴇXPERIMENT XXVI. 6 kilo dog. Injection of Gelatinpeptone (L). 0.6 gram per kilo. One eighth the original size.
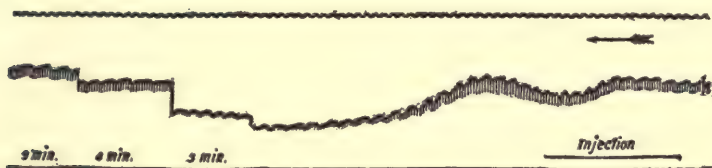
EXPERIMENT XXIX *a*. 15 kilo dog. Injection of Deuterogelatose (K) 0.7 gram per kilo, preceding injection of heteroalbumose. (Cf. tracing Exper. XXIX *b*.) One eighth the original size.
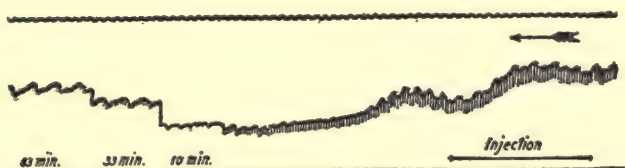
EXPERIMENT XXIX *b*. 15 kilo dog. Cannula in thoracic duct. Injection of Heteroalbumose (H). 0.1 gram per kilo after previous injection of 10.5 grams deuterogelatose (K). (Cf. tracing of Exper. VI.) One eighth the original size.

Influence on blood coagulation.— The older literature on the influence of so-called "peptone" on blood coagulation is too familiar to physiologists to require detailed consideration here. Briefly stated, the researches * made have demonstrated (*a*) that "peptone" does not of itself possess any clot-preventing power, *i. e.*, the action is an indirect one; and (*b*) that accordingly the clot-preventing power must be attributed to some substance formed within the organism and possessing a peculiar influence on the blood.   Furthermore, it seems probable that the peculiar substance is not a transformation product of the "peptone" itself, but is rather a result of other changes taking place in the body.† More recently, through the researches of Contejean,‡ Gley, and Pachon,§ and especially through the researches of Delezenne,‖ the seat of formation of the anti-coagulating substance has been delegated to the liver, and the important rôle which the leucocytes play in this action has been demonstrated by Delezenne (1898).   The explanation formulated at present is as follows: It has long been known that the intravenous injection of "peptone" leads to a pronounced disintegration of leucocytes. Delezenne assumes that this process is accompanied by the liberation of two classes of products, the one accelerating and the other inhibiting blood coagulation.   As types of such bodies, the researches of Lilienfeld ¶ and others have indicated nucleoproteids, which give rise to intravascular clotting, and histon, a peculiar peptone-like body occurring in leucocytes in the compound nucleo-histon.   When these two classes of compounds (clot-retarding and clot-accelerating substances) are

* Schmidt-Mülheim, Archiv f. Physiol. 1880, p. 30; Fano, Ibid., 1881, p. 277; Pollitzer, Journal of Physiology, 1886, vii, p. 283; Campbell, Studies from the Biological Laboratory, Johns Hopkins University, 1887, iv, p. 1; Grosjean, Archives de biologie, 1892, xii; Ledoux, Ibid., 1896, xiv, p. 63.

† Cf. Campbell, loc. cit.

‡ Contejean, Archives de physiologie, 1895.

§ Gley and others, Ibid.   It is to be noted, however, that Starling (Journal of Physiology, 1895, xix, p. 15) has not been able to confirm part of these experiments.

‖ Delezenne, Archives de physiologie, 1897, p. 646; 1898, p. 508.

¶ Lilienfeld, Zeitschr. f. physiol. Chem. 1895, xx, p. 89.

formed incidental to the breaking down of the leucocytes, the liver in its protective rôle preserves the organism from destruction through intravascular clotting by combining with the clot-accelerating substance. A condition thus results in which the blood loses completely or partially its power of coagulating on withdrawal from the vessels. The relative rôle of liver and leucocytes is demonstrated by the fact that if a solution of peptone is passed through an excised dog's liver a fluid results which readily produces " peptone " effects when injected into the circulation of a dog. If, however, the liver is previously freed from blood by lavage through the vessels no clot-retarding fluid can be obtained in the manner described. Delezenne's theory therefore implies the existence of two substances antagonistic in their influence on blood coagulation, — a condition which Spiro and Ellinger * had previously regarded as probable. These latter investigators observed that the injection of acids or of antipeptone overcomes the clot-retarding power of albumoses, while Dastre and Floresco † had previously called attention to the increased alkalinity of "peptone plasma" and the influence of neutralization in inducing coagulation. Lastly, there is evidence that so-called " peptone " may under some conditions hasten the coagulation of the blood. Thus, Thompson ‡ has pointed out that with doses of Witte's " pepton " above 2 centigrams per kilo coagulation is retarded. With doses from 7 milligrams to 2 centigrams per kilo coagulation may be considerably accelerated.

Our experiments on this subject were intended to throw light not so much upon the direct mechanism by which the variations in clotting power are produced, as upon the relative effectiveness of various proteid and albuminoid products. A large number of substances, for the most part extracts of various animal tissues or organs, are known to have the power

* Spiro and Ellinger, Zeitschr. f. physiol. Chem., 1897, xxiii, p. 121.

† Dastre and Floresco, Archives de physiologie, 1897, p. 216. Also Floresco, Ibid., 1897, p. 777.

‡ Thompson, Journal of Physiology, 1896, xx, p. 455.

of retarding blood coagulation either *in vivo* or *in vitro*, or both. Grosjean observed that the proteoses from blood-fibrin retard or even prevent coagulation indefinitely. This action is not produced *in vitro*.* True peptone, he found, never acts as vigorously as the proteoses, and confines its influence to a temporary retardation. In either case the lack of coagulation is very rapidly manifested. Thompson † has recorded similar results with purified peptone. Deuteroproteose gave him inconstant results, while antipeptone hastened blood coagulation, in conformity with the observations of Spiro and Ellinger.‡ Arthus and Huber § have found that much larger doses of gelatoses (2 grams) and caseoses (1.5 grams) per kilo are necessary than in the case of fibrin-proteoses to bring about an absence of coagulation. Lastly, a few experiments have been reported on the action of the albumoses and peptone formed by papaïn digestion. ‖

· The accompanying table, giving the results of our own experiments, shows the time required for the complete coagulation of 2 c.c. blood withdrawn from the femoral or carotid artery at various intervals, expressed in minutes or hours. The normal clotting-time given is in every case the average of two or more observations previous to the injection of the substance under investigation.

The data presented show (1) That in doses of 3 decigrams per kilo of body-weight the albumoses, both hemi and anti, irrespective of the mode of formation, immediately cause suspension of coagulation for at least twenty-four hours, even in blood withdrawn one or two hours after the injection. (Exper. I–VII.)

(2) Antialbumid produces retardation of clotting, the effect being far less marked than in the case of even smaller doses of albumoses, and furthermore requiring some time to

* Cf. Campbell.

† Thompson, Report of Secretary, loc. cit.

‡ Spiro and Ellinger, Zeitschr. f. physiol. Chem., 1897, xxiii, p. 121. See also the older experiments of Pollitzer and of Fano with tryptone.

§ Arthus and Huber, Archives de physiologie, 1896, p. 865.

‖ Chittenden, Mendel, and McDermott, Amer. Jour. Physiol., 1898, i, p. 255.

TABLE SHOWING EFFECTS UPON THE COAGULATION OF THE BLOOD.*

Experiment.	Weight of Animal; kilos.	Grams per kilo Injected.	Nature of the Substance Employed.	Normal Coagulation.	Just after Injection.	5 minutes after.	15 minutes after.	30 minutes after.	1 hour after.	1½ hours after.	2 hours after.
I.	8.8	0.30	Antialbumoses (B) . .	6	8	—	—	—	—	20	—
II.	6.2	0.30	" " .	6	16	—	—	20h	. .	20	10
III.	11.3	0.30	Antialbumoses (C) . .	2	—	—	—	—	—	—	—
IV.	6.0	0.30	" " .	12	—	—	—	—	—	—	—
V.	18.0	0.30	Hemialbumoses (G) .	2	16h	—	—	—	—	—	—
VI.	7.5	0.18	Heteroalbumose (H) .	4	—	—	—	—	—	—	—
VII.	9.8	0.20	" " .	4	—	—	—	—	—	. .	. .
VIII.	7.0	0.30	Antialbumid (A) . .	6	6	14h	1½h	1½h	45	. .	. .
IX.	6.0	0.30	" " . .	2	13	16h	—	16h	. .	. .	. .
X.	5.4	0.30	" " . .	6	40	2h	30	30	7	. .	. .
XI.	7.6	0.14	" " . .	5	30	16	—	16h	16h	. .	. .
XII.	14.0	0.15	" " . .	4	57	70	27	17	9	. .	. .
XIII.	30.0	0.03	" " . .	3	9	16	25	45	14	. .	. .
XIV.	8.4	0.17	" " . .	1	6	4	8	13	6	9	8
XV.	6.8	0.07	Antialbumid (F) . .	3	4	9	4	5	. .	. .	. .
XVI.	9.0	0.30	Antipeptone (D) . .	3	3	8	2	8	3	. .	. .
XVII.	6.5	0.30	" " . .	5	5	8	10	6	10	. .	. .
XVIII.	9.0	0.30	" " . .	4	4	5	5	4	3	. .	. .
XXXI.	4.5	0.45	" " . .	2	4	3	4	10	6	. .	. .
XIX.	11.2	0.30	Hemipeptone (I) . .	9	1	3	3	4	5	. .	. .
XX.	6.5	0.30	" " . .	4	2	3	4	9	7	. .	. .
XXI.†	13.5	0.50	" " . .	8	3	4	6	. .	2	. .	. .
XXII.	6.4	1.00	" " . .	4	4h	—	—	—	—	—	. .
XXIII.	5.0	0.30	Albumose-like body (M)	9	. .	6	35	37	20	14	. .
XXIV.	5.0	1.50	Protogelatose (J) .	10	4	5	6	4	. .	. .	. .
XXV.	4.2	0.30	Gelatin-peptone (L) .	4	3	2	3	4	5	. .	. .
XXVI.	6.0	0.60	" " .	3	2	2	4	4	3	. .	. .
XXVII.	21.0	0.35	Deuterogelatose (K) .	2	2	2	2	. .	1	. .	. .
XXVIII.	7.0	0.50	" " .	3	3h	3h	. .	20	8	2	. .
XXIX.†	15.0	0.70	" " .	3	4	2	8	2	2	2	. .
XXX.	6.5	1.00	" " .	5	2h	—	—	3h	15	4	. .

reach its maximum effect, the results varying in proportion to the dose employed.   (Exper. VIII–XV.‡)

* The figures in the table denote the number of minutes before coagulation, except when followed by h, when they denote hours.   All samples marked — were still fluid after 24 hours.

† The lack of any noticeable action upon the time of coagulation in these two experiments is due to the fact that the lymph was withdrawn through a cannula in the thoracic duct.   See under *lymph-formation* for explanation.

‡ With *antialbumid* (E) *i. e.,* a more insoluble form of the substance separating as a gelatinous coagulum when antialbumid (A) was subjected to pan-

(3) Both hemi- and antipeptone when introduced into the circulation in doses up to 0.5 gram per kilo cause no retardation or even acceleration of coagulation. Larger doses of hemipeptone * (1 gram per kilo), however, act more like the albumoses. (Exper. XXII.)

(4) Protogelatose, as formed by gastric digestion, tends to hasten coagulation even when introduced in large doses.† Large doses of deuterogelatose retard coagulation quite noticeably, our results agreeing in a general way with the observations made by Arthus and Huber ‡ with mixed gelatoses and gelatin-peptone.§ True gelatin-peptone entirely free from gelatoses tends to accelerate slightly the coagulation of the blood.

Independence of arterial pressure and coagulation phenomena. — The results of the present experiments lend weight to the view that the characteristic fall of arterial pressure and the accompanying lack of coagulability are entirely independent phenomena. The former is doubtless due to a direct and

creatic digestion, some peculiar results were obtained which are not included in our table. Thus, in one experiment a dog weighing 5.2 kilos received an injection of 25 c.c. of a solution of this antialbumid in weak ammonia (containing 0.29 gram of the dry antialbumid). Three blood samples taken before the injection clotted in two minutes, and arterial pressure stood at 90 mm. of mercury. One minute after the injection blood-pressure fell to 10 mm. and two minutes later the animal was dead. The heart and larger blood vessels were opened and the blood was found to be clotted solid. A like result was obtained with another dog of the same body-weight, by using a somewhat larger dose of the antialbumid, namely 0.58 gram. In a third experiment, with a dog of 13.6 kilos, using 0.35 gram of antialbumid as the total amount injected, there was a marked fall of arterial pressure, but no effect on blood coagulation. Lastly, with a fourth dog of 16 kilos having a cannula in the thoracic duct, the injection of the antialbumid (0.29 gram in 25 c.c. fluid) led to a fall of arterial pressure, a retardation of 10–12 minutes in the coagulation of the blood, a cessation of the flow of urine from the kidneys, with a small increase in the flow of lymph.

* Cf. the experiments on papaïn-peptone by Chittenden, Mendel, and McDermott, Amer. Jour. Physiol. 1898, i, p. 271.

† Cf. Dastre and Floresco, Archives de physiologie, 1896, p. 402; also Floresco, Ibid., 1897, p. 777.

‡ Arthus and Huber, Archives de physiologie, 1896, p. 857.

§ With reference to the peculiar results obtained in Exper. XXXI see the section on lymph.

immediate action of the peptones and proteoses on the neuro-muscular apparatus of the vessels, while the latter phenomenon stands in no causal relation to the arterial fall. Thus we have observed in many experiments an entire absence of diminished coagulability, notwithstanding a pronounced fall in pressure. (Cf. experiments on peptone, gelatoses, and gelatin-peptone.)[*] Ledoux [†] has arrived at a similar conclusion, and Thompson [‡] has found that a dose of Witte's "pepton" entirely insufficient to retard coagulation may still lower blood-pressure, — as Fano [§] had previously found to be the case in general with acid-albumin. Finally, Dastre and Floresco [‖] have quite recently demonstrated that intravenous injections of bile may entirely prevent the action of proteoses on blood coagulation without interfering with the fall of arterial pressure.

Immunity. — Since the experiments of Schmidt-Mülheim [¶] and more particularly of Fano,[**] it has been known that an intravenous injection of so-called "peptone" (propeptones) which suffices to render the blood non-coagulable for a time apparently confers upon the animal a degree of immunity against subsequent injections of the "peptone." For example, if after the return of normal coagulability to the blood a second injection of albumoses is made, the latter now fails to deprive the blood of its ordinary extravascular clotting power. Furthermore, Fano demonstrated that an injection of tryptone (antipeptone from pancreatic digestion) which is without influence on the coagulation of the blood, nevertheless may render the dog immune against subsequent injections of albu-mose-peptone. Spiro and Ellinger [††] have likewise found that injection of anti-peptone has an antagonistic influence on the action of Witte's "pepton" on blood coagulation. Grosjean [‡‡]

[*] See also Arthus and Huber, loc. cit., p. 861.

[†] Ledoux, Archives de biologie, 1896, xiv, p. 63.

[‡] Thompson, Journal of Physiology, 1896, xx.

[§] Fano, Archiv f. Physiol., 1881, p. 277.

[‖] Dastre and Floresco, Comptes rendus de la soc. de biologie, 1898, p. 458.

[¶] Schmidt-Mülheim, Archiv f. Physiol., 1880, p. 30.

[**] Fano, Archives ital. de biologie, 1882, ii, p. 146.

[††] Spiro and Ellinger, Zeitschr. f. physiol. Chem., 1897, xxiii, p. 137.

[‡‡] Grosjean, Archives de biologie, 1892, xii.

in his comparative study of the physiological action of pro-peptone and peptone reached the following conclusions: A second injection of propeptone is without effect on blood co-agulation if it be carried out on an animal (dog) in which the effect of a previous injection has just disappeared. This is true, says Grosjean, even when the second injection is made with a large dose of the propeptone. The immunity acquired persists, for the most part, about twenty-four hours after the primary return of coagulability, when the blood again becomes coagulable; the time of coagulation not only returns to the normal but may even be shortened. With reference to purified peptone Grosjean concludes that the injection of this substance may likewise confer immunity against subsequent injections of both peptone and propeptone.

Gley and Le Bas [*] have also carried out a series of experi-ments on the behavior of the blood with reference to its coagulability after repeated injections of Witte's "pepton." As a result of their systematic study on a large number of dogs they conclude that in general the period of time during which the blood remains non-coagulable after a rapid injection of proteoses increases with the dose of proteoses employed per kilo of body-weight. Furthermore, the length of time during which immunity to subsequent injections persists also increases with the original dose. Thus, absolute immunity persists after return of the blood to its normal coagulability, about fifteen minutes for 0.01 gram per kilo, and about five hours for a dose of 0.3 gram per kilo.[†] The investigations of Gley and Le Bas thus fail to confirm current statements, namely, that a preliminary injection of proteoses may confer immunity for twenty-four hours toward subsequent injections.

Arthus and Huber [‡] observed that preliminary injections of gelatoses and caseoses were effective in preventing the anti-coagulating effect of subsequent large doses of the same

---

[*] Gley and Le Bas: Archives de physiologie, 1897, p. 848. The experi-ments of Contejean (Ibid., 1895) are not referred to above since they have only an indirect bearing on this subject.

[†] Gley and Le Bas, loc. cit., p. 860.

[‡] Arthus and Huber, Archives de physiologie, 1896, p. 857.

TABLE OF RESULTS BEARING UPON SO-CALLED "PEPTONE" IMMUNITY.

Experiment.	Weight of Animal; kilos.	First Injection. Grams per Kilo Injected.	First Injection. Nature of the Substance Employed.	Time between First and Second Injection.	Second Injection. Grams per Kilo Injected.	Second Injection. Nature of the Substance Employed.	Coagulation of the Blood. Prior to Second Injection.	Coag. after 5 minutes.	Coag. after 15 minutes.	Coag. after 30 minutes.	Arterial Pressure. Prior to Second Injection.	Art. after 5 minutes.	Art. after 15 minutes.
I.	8.8	0.3	Antialbumoses (B).	2½h	0.3	Witte's peptone.	10	20	30	..	110	60	100
VIII.	7.0	0.3	Antialbumid (A).	50	0.3	" "	45	..	..	..	90	22	..
X.	5.4	0.3	" "	2h	0.3	Antialbumid (A).	0	1h	..	..	130	120	150
X.	..	..	" "	20	0.3	Witte's, 3d injection.	..	30	..	..	150	75	135
XV.	6.8	0.07	Antialbumid (F).	45	0.3	Witte's peptone.	5	10	..	..	100	25	40
XVI.	9.0	0.3	Antipeptone (D).	1½h	0.25	" "	8	1½h	—	27	160	50	120
XX.	6.5	0.3	Hemipeptone (I).	1h	0.1	Heteroalbumose (H).	7	5	4	8	180	55	75
XXI.	13.5	0.5	" "	2½h	0.2	" "	2	8	2	..	145	85	110
XXIV.	5.0	1.5	Protogelatose (J).	6h	0.1	" "	4	..	—	..	180	55	100
XXVI.	8.0	0.6	Gelatin-peptone (L).	1½h	0.3	Witte's peptone.	2	4	—	..	115	30	60
XXIX.	15.0	0.7	Deuterogelatose (K).	2½h	0.1	Heteroalbumose (H).	2	5	6	..	160	60	80

NOTE. — Figures in the table referring to time denote minutes, except when followed by h, when they denote hours. Under arterial pressure, the figures denote millimeters. Under coagulation of blood, samples marked — were still fluid twenty-four hours after the experiment. The numbers of the experiments correspond with those given in the preceding tables.

substance. A second fall of blood-pressure, however, was not prevented. Grosjean has pointed out that successive doses of both proteoses and peptones produce successively a less marked fall of arterial pressure, and a second or third injection may even be without effect on this function, — a condition attributed to a degree of immunity acquired by the preliminary injection. It is to be noted, however, that in Grosjean's experiments the injections were undertaken at relatively brief intervals.

In the preceding table are presented protocols from a few of our own experiments bearing on the problem of peptone-immunity. The data presented are somewhat incomplete and fragmentary, still a few facts are clearly demonstrated. Thus it is seen that the injection of active proteoses, such as pure heteroalbumose or the mixed proteoses present in Witte's " pepton," within an hour or two after injection of the substances enumerated in the table fails to develop the characteristic clot-preventing power of the blood. There is to be sure some slight retardation of coagulation, thus indicating that the so-called immunity is by no means complete; but the effect produced by the later injections is very slight indeed as compared with the ordinary action of these products. Experiments XX, XXII, XXIX, XV, and I, afford evidence of the truth of this assertion. In other cases, as in Experiments XVI and XXVI, the immunizing effect is manifested by a delay in the appearance of retarded coagulation. Thus in the two experiments just referred to, a considerable period elapsed before the blood withdrawn from the vessels assumed the characteristics of " peptone-blood." It would almost seem as if the results indicated a deficiency of the clot-preventing substances in the body after the preliminary injection, and that the latter are only *slowly* produced under the influence of the second injection.

With reference to the effects of a second injection of proteoses on arterial pressure our results clearly show that mean arterial pressure is lowered somewhat by the second injection, thus indicating that immunity in this direction is by no means complete. That the first injection, however, does give rise

to some degree of immunity is clearly shown by the fact that the second fall of pressure is noticeably less than the first one, and by the fact that the return of pressure to the normal follows somewhat more rapidly than after the first injection. Our results thus confirm, in part, Grosjean's observations, and extend them by demonstrating that the immunity acquired is by no means characteristic of specific products *injected*, but rather due to a specific reaction produced in each case within the organism itself. Delezenne * has arrived at a similar conclusion from quite different observations. Lastly, Dastre and Floresco † have recently shown that the power of conferring immunity to " peptone " injections is common to a large number of substances, *i. e.*, urine, bile, salts of iron, calcium chloride, etc.

Influence on lymph-flow, etc. — Since Heidenhain's ‡ masterly researches on the action of lymphagogues there has been, so far as we recall, no attempt to compare the lymphagogic action of the individual proteoses and peptones. It is well known that various substances which prevent clotting of the blood simultaneously increase the flow of lymph from the thoracic duct; the substances studied, however, have been for the most part merely extracts of various organs and tissues (leech-extract, extracts of crayfish and mussels). A systematic study of the active constituents of these extracts would be of great interest.

The following table contains the results of several of our experiments on the action of certain specific proteid and albuminoid derivatives on the flow and composition of the lymph. All the animals experimented on were dogs, and were deprived of food for 24–36 hours prior to the experiment.

The results of our experiments justify the following conclusions:

(1) Antialbumid, hemipeptone, heteroalbumose, and deuterogelatose may act as true lymphagogues. This is demon-

* Delezenne, Archives de physiologie, 1897, pp. 657–659.

† Dastre and Floresco, Comptes rendus de la Soc. de Biologie, 1898, p. 457

‡ Heidenhain, Archiv f. d. ges. Physiol., 1891, xlix, p. 216.

TABLE SHOWING INFLUENCE ON LYMPH-FLOW, ETC.*

Experiment.	Time.	Lymph Flow in 10 min. (c.c.)	Total Solids of Lymph. (per cent.)	Ash of Lymph. (per cent.)	Clotting Time of Lymph. (min.)	Clotting Time of Blood. (min.)	Arterial Pressure. (mm. Hg)	Urine Flow in 30 min. (c.c.)
Antialbumid (A). [Exper. XII.] Dog of 14 kilos. Injection of 0.15 gram per kilo body-weight.	Immediately before injection .	1.2	4.90	0.85	10	4	140	10
	Immediately after injection .	10.0	6.73	0.85	?	57	90	:
	One half hour after injection .	3.0	6.10	—	?	17	180	:
Hemipeptone (I). [Exp. XXI.] Dog of 13.5 kilos. Injection of 0.5 gram per kilo body-weight.	Immediately before injection .	4.5	4.47	0.87	10	3	130	4.0
	Immediately after injection .	17.0	5.60	0.86	180	3	40	15.0
	One half hour after injection .	5.0	4.81	—	1	4	130	:
HETEROALBUMOSE (H).† 0.2 gram per kilo injected; 24 hours after the injection of hemipeptone.	Immediately after second injection .	10.5	—	—	—	3	85	None.
Deuterogelatose (K). [Exper. XXVII.] Dog of 21 kilos. Injection of 0.35 gram per kilo body-weight.	Immediately before injection .	4.0	5.77	0.85	30	2	115	1.0
	Immediately after injection .	8.6	5.60	0.89	15	2	115	10.0
	One half hour after injection .	4.7	4.77	—	15	1	120	:
Deuterogelatose (K). [Exper. XXIX.] Dog of 15 kilos. Injection of 0.7 gram per kilo body-weight.	Immediately before injection .	8.0	6.58	0.92	10	3	130	5.0
	Immediately after injection .	15.0	7.78	0.93	90	4	60	15.0
	One half hour after injection .	7.2	6.24	—	20	2	150	7.0
HETEROALBUMOSE (H).† 0.1 gram per kilo injected; 24 hours after the injection of deuterogelatose.	Immediately after second injection .	15.0	7.85	—	50	6	60	None.

Heteroalbumose (H). [Exper. VII.] A dog of 9.8 kilos received an injection of 0.2 gram per kilo body-weight. This was followed by a pronounced fall of arterial pressure; the blood samples taken one hour after the injection failed to clot in twenty-four hours. Owing to an accident to the cannula the lymph-flow could not be measured. There was, however, a pronounced increase after the injection.

* The experiments are numbered to correspond with the numbers used in the other tables.   † Second injection.

strated not only by the marked increase produced in the flow of lymph from the thoracic duct, but likewise by the characteristic changes resulting in the composition of the fluid, namely, increase in total solids, the ash-content remaining constant.* In every case where an increased flow of lymph occurred, the fluid grew slightly reddish in color and its clotting was markedly delayed, — two phenomena characteristic of " lymphagogic " lymph.

(2) In every case where the lymph was thus diverted from the general circulation the clotting time of the blood was only slightly, if at all affected by the injection, while the coagulability of the lymph was delayed. This agrees with the observations of Gley and Pachon,† who announced that the ligature of the lymphatics of the liver prevents the ordinary clot-retarding action of albumose injections in dogs; and with the observations of Spiro and Ellinger, similar to our own, on dogs with the thoracic duct cannulized.‡ In other words, the observations lend favor to the view that the anti-clotting substance is formed in the liver and reaches the blood mainly through the lymph.

(3) A second injection gives rise to a marked renewal of lymph-flow, independent of any action on blood coagulation. The two Experiments XXI and XXIX do not, however, permit the immediate conclusion that the lymphagogic action and clot-preventing phenomena are entirely independent,§ inasmuch as the diversion of the lymph from the circulation may prevent the anti-coagulating substance from reaching the blood.|| Since some dogs are naturally immune to albu-

* Cf. Heidenhain, loc. cit.

† Gley and Pachon, Comptes rendus de l'Acad. des Sciences, 1895, p. 383 ; Archives de Physiologie, 1895, p. 711 ; Comptes rendus de la Soc. de Biologie, 1895, p. 741 ; Ibid., 1897. Cf. also Starling, Journal of Physiology, 1895, xix, p. 15 ; Delezenne, Archives de Physiologie, 1896, p. 657.

‡ Spiro and Ellinger, Zeitschr. f. physiol. Chem., 1897, xxiii, p. 135.

§ Cf. Starling, Journal of Physiology, 1893, xiv, p. 140; also Gley and Pachon, Archives de Physiologie, 1897, p. 861.

|| Asher and Barbèra (Zeitschr. f. Biologie, 1898, xxxvi, p. 154) have put forward the opinion that lymphagogues are substances which stimulate the activity of various organs and especially glands. In agreement with this

mose injections, no far-reaching conclusions can be drawn from the data at hand.

(4) Ordinary doses of deuterogelatose (0.3 gram per kilo) give rise to no marked lymphagogic action, large amounts being required to produce characteristic effects. This corresponds with the other observations made on the physiological action of this substance. With heteroalbumose, lymphagogic action is as strongly marked as the action on blood coagulation and arterial pressure already noted.

(5) Previous injection of hemipeptone or deuterogelatose is entirely without influence on the lymphagogic action of heteroalbumose; the latter producing a marked increase in the flow of lymph when injected a short time after the action of the primary substances has ceased.

Influence on the flow of urine, etc. — In conformity with previous observations, our results, shown in the following table, lead to the conclusion that the albumoses, owing to their marked influence in lowering blood-pressure, tend to cause a complete cessation of the flow of urine. With heteroalbumose, however, the results seemingly justify a somewhat different conclusion. Thus, in three experiments (**XXI**, **XXIV**, and **XXIX**), some hours after the animals had received injections of substances which act as diuretics and lymphagogues, an injection of heteroalbumose was given. This resulted in a complete stoppage of the flow of urine; not a drop of urine was obtained within the half hour or more after this injection, *although the fall of arterial pressure was comparatively slight and transitory.* Hence, it follows that heteroalbumose, unlike the other albumoses, must exert some influence upon the action of the kidneys independent of blood-pressure; possibly, a specific influence upon the secretory activity of the epithelial cells. In any event heteroalbumose may check the formation of urine even when arterial pressure is not materially affected. On the other

view they find that "peptone" is an active "cholagogue." Starling and others, however, have questioned this conclusion. See Proceedings of Fourth International Physiological Congress. Centralbl. f. Physiol., 1898, xii.

hand, as already pointed out, heteroalbumose stimulates in marked degree the flow of lymph. (See preceding table on lymph-flow.)

Antialbumid acts much like ordinary albumoses in checking the secretion of urine, but if comparison is made, by reference to the table showing arterial pressure, of the influence on renal secretion and mean arterial pressure, as in Experiments VIII, X, and XI, it will be seen that the inhibitory effect on the secretory action of the kidneys is considerably greater than would naturally be expected from the short period of diminished arterial pressure. This fact obviously suggests a specific action on the part of antialbumid upon the kidneys independent of pressure.

On the other hand, large doses of hemipeptone, protogelatose, and deuterogelatose have a marked diuretic effect, increasing not only the volume of urine secreted in a given time, but likewise the content of solid matter, as shown by the very decided increase in specific gravity.* Coincident with the diuresis it will be observed that mean arterial pressure is slightly raised under the influence of the substance. The marked rise in the specific gravity of the urine, noticed after the injections, is associated mainly with the rapid excretion of the substance thrown into the circulation. As has been previously noted † in this connection, a primary proteose introduced into the blood is eliminated, in part at least as a secondary proteose. In other words, the primary body undergoes hydration in the kidney ‡ or elsewhere, and is excreted through the urine in slightly altered form. Thus, in Experiment XXIV with protogelatose not only was the rate of flow of the urine more than doubled, but the specific gravity rose from 1021 to 1061. Examination of the urine showed the presence of a large amount of gelatose, but this was mainly deuterogelatose.

---

* See Chittenden, Science, N. S. v, June 11, 1897, p. 902; also Thompson, Journal of Physiology (Proceed. Physiol. Soc.), 1898, xxii, p. xi.

† Neumeister, Zeitschr. f. Biologie, 1887, xxiv, p. 272; also Chittenden, Mendel, and McDermott, Amer. Jour. Physiol., 1898, i, p. 266.

‡ Neumeister, loc. cit.

TABLE SHOWING EFFECTS UPON URINARY SECRETION.

Experiment.	Weight of Animal.	Grams per kilo injected.	Nature of the Substance Injected.	Urine in 30 mins. before injection.		Urine in 30 mins. after injection.		Remarks.
	kilos.			Vol c.c.	Sp. Gr.	Vol c.c.	Sp. Gr.	
VIII.	7.0	0.3	Antialbumid (A)	19.2	...	2.6	...	Strong biuret reaction.
X.	5.4	0.3	" " "	7.2	...	0	...	" " "
XI.	7.6	0.14	" " "	7.0	...	2.9	...	" " "
XVI.	9.0	0.3	Antipeptone (D)	...	...	30.0	...	
XVII.	6.5	0.3	" " "	6.7	...	10.5	...	
XVIII.	9.0	0.3	" " "	4.2	1026	43.5	1016	
XIX.	11.2	0.3	Hemipeptone (I)	46.2	1031	38.0	1036	Urine almost black.
XX.	6.5	0.3	" " "	4.0	...	4.0	...	No biuret; normal color.
XXI.	18.5	0.5	" " "	4.0	1067	15.0	1042	No biuret; dark color.
XXII.	6.4	1.0	" " "	17.5	1035	55.0	1035	" " " "
XXIII.	5.0	0.3	Albumose-like body (M)	2.5	...	0	...	" " " "
XXIV.	5.0	1.5	Protogelatose (J)	13.8	1021	82.0	1061	Dark color. Contained deuterogelatose and peptone.
XXV.	4.2	0.3	Gelatin-peptone (L)	...	...	25.0	...	No biuret; dark color.
XXVI.	6.0	0.6	" "	15.0	1045	10.0	1045	" "
XXVII.	21.0	0.35	Deuterogelatose (K)	1.0	...	10.0	...	
XXVIII.	7.0	0.5	" "	8.0	1046	10.0	1093	Dark urine. Contained deuterogelatose and peptone.
XXIX.	15.0	0.7	" "	5.0	1062	15.0	1098	
XXX.	6.5	1.0	" "	2.1	...	4.5	...	

Further, on saturating the urine with ammonium sulphate under appropriate conditions for removal of all the gelatoses, and testing the filtrate by the biuret test, a distinct reaction for true gelatin-peptone was obtained. Five and a half hours after the injection the urine still contained an appreciable amount of gelatose. After injection of deuterogelatose the urine was likewise found rich in gelatin-peptone, while some unaltered gelatose was also found. This excretion of gelatoses through the urine as gelatoses and gelatin-peptone was quite marked, and we were able to separate from such urines several grams of fairly pure gelatose, thus showing that the elimination through this channel was not inconsiderable. If it is true, as suggested by Neumeister, that the hydration of a primary proteose to secondary proteose and the hydration of the latter to true peptone in these cases is due to the presence of pepsin in the kidneys acting in conjunction with acid temporarily present, then our results testify to the extreme vigor of such action, and indirectly suggest the presence of considerable active enzyme. Whether this hydrolytic action is truly due to the presence of an enzyme our results afford no answer. In this connection it will be remembered that Hofmeister* observed that when amphopeptone was injected subcutaneously, four-fifths of the substance appeared in the urine unaltered. Neumeister † likewise noted that amphopeptone was excreted as such through the urine. In a paper from this laboratory,‡ however, attention was called to the fact that peptone formed through the action of papaïn was much less markedly eliminated through the urine than deuteroalbumose; this statement being based upon the much weaker biuret reaction given by the urine. In conformity with this observation we have noted in the present experiments with hemipeptone and gelatin-peptone that after the intravenous injection of these substances their presence in the urine could

* Hofmeister, Zeitschr. f. physiol. Chem., 1881, v, p. 131.
† Neumeister, Zeitschr. f. Biologie, 1887, xxiv, p. 287.
‡ Chittenden, Mendel, and McDermott, Amer. Jour. Physiol., 1898, i, p. 275.

not be detected by the biuret reaction. Whether this fact is to be taken as an indication of their excretion by the urine in the form of amido-acids, etc., formed through proteolytic action in the kidneys, or whether it is due to decomposition taking place elsewhere, or to a storing up within the body we cannot say.

Observations on the Chemical Nature and General Properties of the Proteid Cleavage Products used in the Preceding Experiments.

Among the many earlier results obtained in the attempt to throw light upon the chemical nature of the proteid molecule those recorded by Schützenberger * were particularly suggestive, especially from a physiological standpoint. The breaking down of the proteid molecule into two equal parts by the action of 3 to 5 per cent sulphuric acid at 100° C. naturally suggested the possible existence of two distinct groups in the mother proteid, — a suggestion which was intensified by the observations of Kühne † that these two cleavage or alteration products behaved quite differently toward alkaline pancreatic juice. Schützenberger through his hydrolysis of coagulated egg-albumin by dilute sulphuric acid obtained an insoluble residue equal to about 50 per cent of the original proteid, to which he gave the name of hemiprotein, while the 50 per cent of soluble matter was represented by a row of substances among which leucin, tyrosin, peptone, and albumoses (as now termed) were conspicuous. As Schützenberger pointed out, it was quite evident that these latter products resulting from this hydrolysis represented a half of the proteid molecule much less stable than that portion of the proteid from which the so-called hemiprotein was derived. This more readily decomposable half of the proteid he considered as represented by a body

* Schützenberger, Bulletin de la Société chimique de Paris, 1875, xxiii, and xxiv.

† Kühne, Verhandl. d. Naturhist.-Med. Vereins zu Heidelberg, 1877, i, p. 236; Kühne and Chittenden, Zeitschr. f. Biologie, 1883, xix, p. 159.

which he termed hemialbumin, and which by continued hydroly-
sis gave rise to peptone and amido-acids. These conclusions
were verified in part, and extended by Kühne in his study of
the action of the proteolytic enzymes upon these two bodies, —
i. e., hemiprotein and hemialbumin. The former was found to
be slowly digestible by pepsin-hydrochloric acid, while by
alkaline pancreatic juice it was eventually transformed in great
part into a peptone without any trace of amido-acids being
formed.* In other words, the substance was remarkably
resistant to that secondary action of the pancreatic enzyme by
which pancreatic proteolysis is especially characterized. On
this account Kühne suggested the name of antialbumid as
more expressive of the general nature of the substance than
the term hemiprotein, and by this name the substance has
generally been known for the past twenty years. The so-
called hemialbumin of Schützenberger was found by Kühne to
be readily digestible in artificial gastric juice, giving rise to
peptone, while, by the action of pancreatic juice, leucin and
tyrosin were formed in abundance. To the hemialbumin of
Schützenberger, Kühne gave the name of hemialbumose,
which prevailed for a time until it was found that this sub-
stance was in reality a mixture of several related substances,
to which the name of albumoses was applied.† Through
these researches, followed by many others that need not be
enumerated here, the view that in the native proteid molecule
there are present two distinct atomic complexes has gradually
gained credence, and as a result the prefixes hemi and anti
have been, and are, widely used to designate inner differences
in the various hydrolytic cleavage products of the proteids,
whether formed by the action of boiling dilute acids or by
the action of proteolytic enzymes. A hemi body has thus
come to mean a substance which under the influence of trypsin
will break down into crystalline amido-acids and other simple
products, while an anti body fails to yield any such simple
products under the action of trypsin. The researches of

---

* Kühne and Chittenden, loc. cit.
† Kühne and Chittenden, Zeitschr. f. Biologie, 1884, **xx**, p. 11.

Neumeister * and others have shown, however, that when this crucial test is applied to the specific proteoses formed in gastric digestion, for example, there is invariably an admixture of the so-called hemi and anti groups; not an equal mixture, — for protoproteose is apparently composed in great part of hemi groups with only a small admixture of anti groups, while heteroproteose is made up chiefly of anti groups, as judged by the behavior of the respective bodies toward trypsin. In the amphopeptone of gastric digestion, on the other hand, we have apparently a commingling in equal proportion of hemi and anti groups, for if this substance is digested for some time with a vigorous pancreatic juice, approximately one half breaks down into crystalline decomposition products, leaving a residue of peptone resistant to the further action of trypsin, *i. e.*, an antipeptone. How far these terms hemi and anti are to be considered as representing real differences in chemical constitution is very uncertain. It may be that the differences are more apparent than real, and that the characteristic behavior toward trypsin is the result of more or less superficial peculiarities. Granting that this is so, the terms still have significance as indicating physiological differences of some moment and are thus of value. Certainly the view at one time suggested that an anti body contained no aromatic groups capable of yielding tyrosin under any conditions is not tenable. To be sure, a pure antipeptone gives little or no Millon's reaction, but antialbumid boiled for some time with 10 per cent sulphuric acid will yield considerable tyrosin, although when acted upon by an alkaline solution of trypsin no tyrosin whatever results. Thus, Dr. Alice H. Albro, working in this laboratory with antialbumid made from coagulated egg-albumin, found on boiling the substance with 10 per cent sulphuric acid for 80 hours that 2.7 per cent of tyrosin was formed, together with considerable leucin. In this connection it will be remembered that Erlenmeyer and Schöffer obtained 1 per cent of tyrosin by boiling egg-albumin with sulphuric acid, while Schützenberger obtained 2.0 per cent by

* Neumeister, Zeitschr. f Biologie, 1887, xxiii, p. 380.

heating egg-albumin at 160–200° C. with baryta water for 4 to
6 days.* Obviously, one cannot draw very sharp comparisons
between these several results, but it is quite clear that antial-
bumid made from coagulated egg-albumin by the action of 4
per cent sulphuric acid at 100° C. yields fully as much tyrosin
on decomposition with a strong mineral acid as the mother
proteid. Hence the difference in the result when these two
substances are treated with active pancreatic juice must be
due, not to the lack of the characteristic aromatic group in the
antialbumid molecule, but rather to some peculiar state of
combination of the atoms or radicles which prevents the for-
mation of tyrosin or other amido-acid through the action of
trypsin. In the case of antialbumid formed by the hydrolytic
action of a dilute acid it might perhaps be claimed that it is
purely an artificial product, and that its resistance to the
secondary action of trypsin is merely the result of some slight
alteration of the original proteid attendant upon the process
of cleavage. As opposed to this idea, however, we have the
well-known fact that the original coagulated albumin placed
directly in alkaline pancreatic juice breaks down step by step
to leucin, tyrosin, etc., leaving at the end a large amount —
perhaps 50 per cent — of a peptone (antipeptone) which
resembles the peptone resulting from the pancreatic digestion
of antialbumid. In other words, there is abundant reason for
believing that the so-called anti group is contained as such in
the native proteid, and that in the cleavage with dilute
sulphuric acid it is split off as antialbumid, while in the pan-
creatic digestion of the native proteid it is isolated as anti-
peptone through conversion of the so-called hemi groups into
amido-acids and nitrogenous bases.

While it is thus possible to isolate with some degree of
purity bodies belonging to or derived from the anti half of the
proteid molecule, less satisfactory is the isolation of the so-
called hemi bodies. From the amphopeptone formed in gastric
digestion, the antipeptone can be separated through destruction
of the hemi groups, but there is no way known of isolating

* See Hermann's Handbuch der Physiologie, v (2), p. 212.

the hemipeptone from this mixture. A method promising better results is the hydrolytic cleavage of the native proteid with dilute acid, accompanied as it is by the splitting off of the anti groups as antialbumid. While this results in the formation of soluble hemialbumoses and hemipeptone, which can easily be separated, it unfortunately happens that the antialbumid formed also undergoes hydration to some slight degree with consequent formation of some soluble antialbumoses and antipeptone. These latter bodies, once mixed with the corresponding hemi products, cannot be separated, and hence are an ever-present impurity. Still, this method affords the only way, at present, of obtaining the so-called hemialbumoses and peptone, and we have had recourse to it as the only available method, understanding, however, that the several products are by no means wholly free from corresponding anti bodies.

As already stated in the first part of this paper, it seemed very desirable in view of the importance attaching to the physiological action of the various digestive products of the proteids to ascertain as definitely as possible the part played by the several components of the ordinary products of proteolytic action. With this end in view we have devoted particular attention to the anti products, and in order to insure their freedom, so far as possible, from the so-called hemi groups we have made use of antialbumid as the mother substance in the preparation of antialbumoses and antipeptone.

Hydrolysis and cleavage of coagulated egg-albumin with formation of antialbumid, etc.—In obtaining sufficient material for our purpose it was necessary to repeat the process several times, but the general line of procedure in each case was as follows: The whites of twelve dozen eggs were coagulated by pouring them slowly into a large volume of boiling water acidified slightly with acetic acid, the coagulum collected on a cloth filter and washed with large quantities of boiling water. It was then pressed as dry as possible and placed in a large flask with 4 per cent sulphuric acid (360 grams of the moist coagulum in 1200 c.c. of the acid). This mixture was then heated

at 100°C. in a large steam sterilizer (Arnold's), with occasional agitation, for 12 to 15 hours, after which the gelatinous anti-albumid was separated from the acid solution (containing the hemi bodies) by filtration through paper and freed from all soluble matter by long continued washing with water. The washing was continued until the wash-water reacted only faintly acid. In some cases the antialbumid was again heated at 100° C. for 12 hours with a fresh lot of 4 per cent sulphuric acid. The crude antialbumid was next warmed at 40° C. for forty hours with a large volume of an active solution of pepsin-hydrochloric acid for the complete removal of adherent hemi bodies or of any unaltered egg-albumin. The residual antialbumid was then filtered off and washed thoroughly with distilled water until the washings were free from chlorine. The product was next dissolved in a solution of 0.5 per cent sodium carbonate, the fluid filtered through paper, and the antialbumid reprecipitated by neutralization with 0.2 per cent hydrochloric acid, after which it was again washed with water until free from chlorine, and lastly with 95 per cent alcohol. This product constitutes *antialbumid (A)*.

In separating the so-called hemialbumoses from the 4 per cent sulphuric acid solution, the clear filtered fluid was neutralized with ammonia and evaporated to a convenient volume, the neutralization precipitate filtered off, and the further concentrated fluid eventually saturated boiling hot with ammonium sulphate in acid, neutral and alkaline reaction according to the method suggested by Kühne.* The precipitate of mixed albumoses was dissolved in water, a little thymol added, and the fluid dialyzed in running water until the sulphate was entirely removed. When the dialysis was completed a small quantity of heteroalbumose was found adherent to the parchment. This was therefore filtered off, washed thoroughly with water, lastly with alcohol, and dried. This product constitutes *heteroalbumose (H)*. Assuming the correctness of Neumeister's statement that heteroalbumose as formed in pepsin-proteolysis is composed mainly of anti groups, it suggests that

---

* Kühne, Zeitschr. f. Biologie, 1892, xxix, p. 1.

the formation of this substance in the hydrolysis with sulphuric acid may be taken as a measure of the extent to which in this hydrolysis anti groups are split off in forms other than antialbumid.   On the other hand, there is perhaps no logical reason why a heteroalbumose might not be formed under this peculiar method of hydrolysis, out of hemi groups mainly.

The above fluid freed by dialysis from ammonium sulphate and heteroalbumose, after filtration from the latter substance, was concentrated to a syrup, precipitated with alcohol, and the precipitate washed thoroughly with hot alcohol and dried. This product constitutes *hemialbumoses* (*G*), and is composed of a mixture of hemiprotoalbumose and hemideuteroalbumose.

The hemipeptone contained in the ammonium sulphate-saturated fluid after separation of the mixed albumoses was obtained as follows.   The fluid was concentrated somewhat, cooled, and the crystals of ammonium sulphate separated, this operation being repeated four times, thus accomplishing the removal of a large amount of the contained sulphate.   During the repeated heating of this solution a small quantity of a dark oily albumose-like substance was obtained, which was filtered off and freed from ammonium sulphate by the method used in the purification of the peptone, *i. e.*, with barium carbonate, etc.   The product so isolated constitutes the *albumose-like body* (*M*).   The solution was next treated with barium hydroxide until the larger portion of the sulphate was precipitated, after which the last portions of the ammonium salt were removed by warming the solution with pure barium carbonate.   The filtered fluid was then treated with very dilute sulphuric acid, drop by drop, until the last trace of dissolved barium was removed and the filtrate concentrated to a small volume, precipitated with alcohol, and the precipitate washed repeatedly with boiling alcohol.   This product constitutes *hemipeptone* (*I*).

Formation of antialbumoses by the action of pepsin-acid on pure antialbumid. — When antialbumid is warmed at 40° C. with an active solution of pepsin-hydrochloric acid (0.2 per cent HCl) there results a slow but gradual digestion of the

proteid with formation of soluble antialbumoses together with a trace of peptone. Using antialbumid (A) as the mother substance, digestion with pepsin-acid was carried on for some days, the undigested residue filtered off, the solution neutralized with sodium carbonate, concentrated, and the albumoses separated by saturation of the boiling solution with ammonium sulphate. The gummy precipitate so obtained was dissolved in water, dialyzed in running water until free from sulphate, then concentrated to a syrup and precipitated with alcohol. The product was a mixture of protoalbumose and deuteroalbumose, and constitutes the preparation called *antialbumoses* (*B*).

Antialbumoses and antipeptone formed by the action of an alkaline solution of trypsin on antialbumid. —Antialbumid (A) was dissolved in 0.5 per cent sodium carbonate and the solution warmed at 40° C. for five days with a purified solution of trypsin,* also in 0.5 per cent sodium carbonate, thymol being added to prevent putrefaction. After the clear solution had been at 40° C. for a few hours it became opaque and soon formed a thick jelly due to the separation of a portion of the antialbumid in a slightly modified form, as has frequently been described in other places.† At the expiration of the five days a large proportion of this insoluble matter had disappeared, but there still remained an appreciable amount of the substance, which was filtered off, thoroughly washed with water, and dried. This constitutes *antialbumid* (*E*.) The alkaline solution containing the albumoses and peptone was neutralized with dilute hydrochloric acid, the slight precipitate removed by filtration, and the fluid concentrated to a convenient volume. The antialbumoses and antipeptone were then separated by ammonium sulphate and isolated exactly as

---

* The trypsin solution was prepared by warming 40 grams of Kühne's dry ox pancreas in 400 c.c. 0.1 per cent salicylic acid solution for 24 hours, making the extract alkaline (0.25 per cent Na_2CO_3) and warming this at 40° C. for 48 hours in the presence of thymol. The extract was next dialyzed for some days from both alkaline and acid (acetic acid) solution, then evaporated to dryness at 40° C. and the residue taken up with a very little cold water This solution, filtered, contains fairly pure trypsin.

† Kühne and Chittenden, Zeitschr. f. Biologie, 1883, xix, p. 166.

described under the head of hemialbumoses and hemipeptone. The antipeptone, however, owing to the presence of sodium chloride, was further purified by dialysis until the chloride was entirely removed. The product is *antipeptone* (*D*).

The antialbumoses, indicated as *antialbumoses* (*C*), were a mixture of proto and deuteroalbumose. This is a matter of some moment chemically, for it is generally assumed that in trypsin-proteolysis primary proteoses are not formed; that the proteid passes directly to deuteroproteose and thence into true peptone, etc.* Further, it is implied in the scheme of pepsin-proteolysis generally accepted by writers on this subject, that antialbumid undergoing gastric digestion is transformed directly into antideuteroalbumose without any intermediate formation of a primary proteose.† This, however, is not the case; certainly not where pure antialbumid is undergoing digestion. Thus, we have observed that in the digestion of antialbumid with both gastric juice and pancreatic juice, the albumoses formed and separated contained a large amount of protoalbumose; the neutral solution of the albumoses yielding a very decided precipitate on saturation with sodium chloride.

The antipeptone (D) formed by the action of trypsin on pure antialbumid was found on analysis to have the following composition, when dried at 110° C.

I. 0.3003 gram substance gave 0.1812 gram $H_2O = 6.70$ per cent H and 0.5502 gram $CO_2 = 49.96$ per cent C.
II. 0.3330 gram substance gave 0.6075 gram $CO_2 = 49.75$ per cent C.
III. 0.2681 gram substance gave by the Kjeldahl method 0.03570 gram N = 13.32 per cent N.
IV. 0.2722 gram substance gave 0.03612 gram N = 13.27 per cent N.
V. 1.0382 grams substance gave by fusion with NaOH ‡ and KNO_3 0.1203 gram $BaSO_4 = 1.59$ per cent S.
VI. 1.0665 grams substance gave 0.0228 gram ash = 2.14 per cent.

* See Neumeister's Lehrbuch d. physiol. Chem., 1897, p. 247.
† See Neumeister, Zeitschr. f. Biologie, 1887, xxiii, p. 391.
‡ Pure sodium hydroxide made from the metal sodium and free from sulphur. The fusion was made in a silver crucible over an alcohol lamp.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

	I.	II.	III.	IV.	V.	Average.
C	51.05	50.82	. . .	. . .	. . .	50.93
H	6.84	. . .	. . .	. . .	. . .	6.84
N	. . .	. . .	13.61	13.56	. . .	13.58
S	. . .	. . .	. . .	. . .	1.62	1.62
O	. . .	. . .	. . .	. . .	. . .	27.03
						100.00

This is the only analysis, so far as we are aware, of an antipeptone prepared from pure antialbumid. Some years ago Kühne and Chittenden * analyzed a preparation of so-called antipeptone prepared by the digestion of antialbumid with alkaline pancreatic juice, but at that time the proteoses had not been discovered and consequently the method of separation made use of did not effect the removal of the antialbumoses; hence, the product analyzed was without doubt a mixture of true antipeptone and antialbumoses. In considering the composition of this product it will be remembered that antialbumid is characterized by a comparatively high content of carbon and a low content of nitrogen (53.79 per cent C and 14.55 per cent N),† hence it is to be observed that in the formation of this peptone, as in all cases of peptone formation, the mother proteid loses an appreciable amount of carbon.‡ Somewhat noticeable in this product is the comparatively high content of sulphur, for peptones as a rule contain a very small amount of this element. There was no sulphur, however, present in the mercaptan form, and the ash contained no sulphate. Neither was there any evidence of the presence of ammonium sulphate. As to the relationship in composition between this antipeptone and the antipeptone resulting from the pancreatic digestion of a native proteid it will suffice here to refer the reader to previous papers on antipeptone.§

* Kühne and Chittenden, Zeitschr. f. Biologie, 1883, xix, p. 169.

† Kühne and Chittenden, loc. cit., p. 167.

‡ See Chittenden, Digestive Proteolysis, New Haven, 1895, p. 70.

§ Kühne and Chittenden, Zeitschr. f. Biologie, 1886, xxii, p. 423; Chittenden, Studies in Physiological Chemistry, Yale University, 1889, iii, p. 100;

Another product to be referred to under the head of anti bodies is *antialbumid* (*F*). This substance was prepared from well crystallized edestin,[*] a globulin obtained from hemp-seed, by heating the substance at 100° C. with 4 per cent sulphuric acid. The gelatinous antialbumid resulting by this process was purified by solution in 0.5 per cent sodium carbonate, etc., and then used for intravenous injection.

Hydrolysis and cleavage of pure gelatin by trypsin in neutral solution. — In this hydrolysis 200 grams of exceptionally pure gelatin prepared from the purified collagen of white fibrous connective tissue (tendons) by Dr. Van Name [†] were available. This material was dissolved in four liters of distilled water, a neutral solution of purified trypsin [‡] added, together with sufficient thymol to prevent putrefaction, and the mixture warmed at 40° C. for four weeks. The object of this long-continued digestion was to insure the formation of as large an amount as possible of true gelatin-peptone, the substance usually described under this name being nothing more than a mixture of gelatoses. Further, digestion was carried on in a *neutral* fluid to obviate the necessity for removal from the resultant products of alkali salts; a process which entails great loss of substance. Careful examination of the digestive mixture at the end of the four weeks showed the complete absence of primary gelatoses. The solution was therefore concentrated and the deuterogelatose separated directly by saturation with ammonium sulphate under the conditions already referred to. The product was purified by dialysis, and eventually precipitated from a concentrated solution by strong alcohol, after which it was repeatedly boiled with alcohol for removal of any soluble extractives and then dried. This product constitutes *deuterogelatose* (*K.*) The yield was **113** grams.

Chittenden and Goodwin, Journal of Physiology, 1891, xii, p. 34; Balke, Zeitschr. f. physiol. Chem., 1896, xxii, p. 248, etc.

    [*] Chittenden and Mendel, Journal of Physiology, 1894, xvii, p. 48.
    [†] Van Name, Journal of Experimental Medicine, 1897, ii, p. 117.
    [‡] See foot-note (p. 315).

The ammonium sulphate-saturated fluid containing the true gelatin-peptone formed by trypsin was freed from the excess of ammonium salt by crystallization, the residue removed by treatment with barium hydroxide and barium carbonate as described in the preparation of hemipeptone, and the true peptone precipitated by alcohol. After thorough and repeated extraction with boiling alcohol the peptone was dried. *Gelatin-peptone* (*L*). It weighed 17 grams. The relative yield of deuterogelatose and true peptone in this long-continued hydrolysis with an active solution of trypsin constitutes striking evidence of the relatively slow production of true gelatin-peptone in trypsin-proteolysis.

The deuterogelatose in aqueous solution gave no precipitate whatever on saturation of the fluid with sodium chloride, neither did any precipitate result on addition of acetic acid to the salt-saturated fluid. Consequently, it must be considered as entirely free from protogelatose.* Tested with potassium hydroxide and plumbic acetate for loosely combined sulphur, both peptone and gelatose gave negative results. Negative results were likewise obtained with Millon's reagent and the xanthoproteic test. With the biuret test, strong solutions of both gelatose and peptone yielded a bright pink color. The composition of these two products is shown by the following results.

ANALYSIS OF DEUTEROGELATOSE (K)†.

I. 0.2811 gram substance gave 0.1705 gram $H_2O = 6.74$ per cent H and 0.5201 gram $CO_2 = 50.46$ per cent C.

II. 0.2895 gram substance gave 0.1769 gram $H_2O = 6.79$ per cent H and 0.5356 gram $CO_2 = 50.45$ per cent C.

III. 0.3195 gram substance gave by the Kjeldahl method 0.05556 gram N = 17.39 per cent N.

IV. 0.2952 gram substance gave 0.05134 gram N = 17.39 per cent N.

V. 0.5166 gram substance gave by fusion with NaOH and KNO_3 0.0126 gram $BaSO_4 = 0.33$ per cent S.

VI. 0.3695 gram substance gave 0.0030 gram ash = 0.81 per cent.

---

* See Chittenden and Solley, Journal of Physiology, 1891, xii, p. 33.

† Dried at 105° C. until of constant weight.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

	I.	II.	III.	IV.	V.	Average.
C	50.87	50.86	. . .	. . .	. . .	50.87
H	6.79	6.84	. . .	. . .	. . .	6.81
N	. . .	. . .	17.53	17.53	. . .	17.53
S	. . .	. . .	. . .	. . .	0.34	0.34
O	. . .	. . .	. . .	. . .	. . .	24.45
						100.00

ANALYSIS OF GELATIN-PEPTONE (L).

I. 0.2984 gram substance gave 0.1797 gram H_2O = 6.69 per cent H and 0.5168 gram CO_2 = 47.23 per cent C.

II. 0.2134 gram substance gave 0.1286 gram H_2O = 6.69 per cent H and 0.3709 gram CO_2 = 47.40 per cent C.

III. 0.2869 gram substance gave by the Kjeldahl method 0.04827 [gram N = 16.82 per cent N.

IV. 0.2168 gram substance gave 0.03661 gram N = 16.88 per cent N.

V. 0.5017 gram substance gave by fusion with NaOH and KNO_3 0.0119 gram $BaSO_4$ = 0.32 per cent S.

VI. 0.5376 gram substance gave 0.0114 gram ash = 2.12 per cent.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

	I.	II.	III.	IV.	V.	Average.
C	48.25	48.42	. . .	. . .	. . .	48.33
H	6.83	6.83	. . .	. . .	. . .	6.83
N	. . .	. . .	17.18	17.24	. . .	17.21
S	. . .	. . .	. . .	. . .	0.33	0.33
O	. . .	. . .	. . .	. . .	. . .	27.30
						100.00

Comparing these results with the composition of the pure gelatin from which the gelatose and peptone were derived we have the following figures:

	Gelatin.*	Deuterogelatose.	Gelatin-Peptone.
C	50.11	50.87	48.33
H	6.56	6.81	6.83
N	17.81	17.58	17.21
S	0.25	0.34	0.33
O	25.24	24.45	27.30

Thus, again we have a forcible illustration of the fact that in both pepsin- and trypsin-proteolysis the formation of true peptone is associated with a marked diminution in the content

* Van Name, loc. cit., p. 124.

of carbon.* The composition of the deuterogelatose, on the other hand, is seen to be essentially identical with that of the gelatin from which it was derived, thus agreeing with the observations previously recorded by Chittenden and Solley † on the composition of the gelatoses formed by gastric and pancreatic digestion.

The protogelatose (protogelatose J) tested intravenously was a specimen prepared by pepsin-proteolysis of a purified commercial gelatin,‡ and had the following composition:

C 49.98, H 6.78, N 17.86, S 0.52, O 24.86, Ash 1.98.

ADDENDUM.

Since this work was completed two exceedingly interesting and important papers on antipeptone have appeared from Kossel's laboratory,§ which have a direct bearing upon the purity of antipeptone formed from blood-fibrin by digestion with an alkaline pancreatic extract. Kutscher's results tend to show that ordinary antipeptone formed from blood-fibrin by pancreatic digestion and purified by the methods ordinarily made use of contains a large proportion of impurities composed in great part of the nitrogenous bases arginin, histidin, and a new body as yet undescribed, together with aspartic acid and some leucin and tyrosin. Assuming this to be true of all antipeptones formed from native proteids by direct digestion with pancreatic juice, it follows that the products of this kind hitherto tested physiologically are open to the suspicion of being unsuitable for experiments of this nature. How far the same criticism will apply to the antipeptone employed by us in the present experiments is uncertain. It would seem, however, that the antipeptone prepared from antialbumid might perhaps be free from this objection or at least contain a far smaller amount of impurity, since in the hydrolysis and

---

* Chittenden, Digestive Proteolysis, New Haven, 1895, p. 71.

† Chittenden and Solley, Journal of Physiology, 1891, **xii**, p. 23.

‡ Chittenden and Solley, loc. cit., p. 28.

§ Kutscher, Zeitschr. f. physiol. Chem., 1898, **xxv**, p. 195; Kutscher, Ibid., 1898, **xxvi**, p. 110.

cleavage of egg-albumin in the preparation of antialbumid the more easily decomposable half of the proteid molecule would be at once eliminated. Consequently in forming antipeptone from pure antialbumid by trypsin-proteolysis it is quite possible that the resultant peptone is free from the objectionable features of ordinary antipeptone, owing to the nature of the mother substance. If such is the case our present experiments possess a double value. In partial justification of the belief that antipeptone prepared from antialbumid is less liable to contain the impurities referred to is the fact that the well known bodies leucin and tyrosin, so common among the products formed in the breaking down of the native proteid with trypsin and usually associated with the nitrogenous bases, are never found in the proteolysis of pure antialbumid. Further in the original analyses of antipeptone made by Kühne and Chittenden,[*] it was found that purification by phosphotungstic acid tended to raise decidedly the content of nitrogen; a fact which Kutscher explains by assuming the more complete precipitation of the nitrogenous bases by this reagent. Thus, in a preparation of fibrin-antipeptone containing 16.58 per cent of nitrogen, precipitation by phosphotungstic acid resulted in raising the content of nitrogen in the substance to 18.28 per cent.[†] In other words, this method of purification led, according to Kutscher, to an increase in the proportion of impurities. This being so, the higher content of nitrogen in an antipeptone is to be looked upon with suspicion. Accepting the truth of this statement, the extremely low content of nitrogen in the antipeptone from antialbumid (13.58 per cent) may perhaps be taken as additional evidence of the freedom of the peptone from the objectionable nitrogenous bases. It is not to be understood from this hypothesis that the anti bodies, antialbumid or antipeptone, will not yield nitrogenous bases on decomposition. The real distinction between a hemi and anti body is based upon the behavior of the substance toward trypsin. As we have already pointed out, antialbumid, which

[*] Kühne and Chittenden, Zeitschr. f. Biologie, 1886, xxii, p. 435.
[†] Kühne and Chittenden, loc. cit., p. 452.

will not yield tyrosin on treatment with trypsin, will yield considerable tyrosin on decomposition with a strong mineral acid at 100° C. So, likewise, these anti bodies may yield a large proportion of the nitrogenous bases on decomposition with a boiling mineral acid while wholly resistant to the action of trypsin.

In this connection it has seemed to us important to ascertain how large a yield of nitrogenous bases can be obtained from antialbumid by decomposition with a mineral acid, and also to compare the results with those obtainable by a like decomposition of a hemi body. For this purpose, 10 grams each of antialbumid, hemialbumoses, and hemipeptone were boiled for ninety-six hours with 40 c.c. of 20 per cent hydrochloric acid with addition of a little stannous chloride. The solutions were then diluted with water, the tin removed by hydrogen sulphide, and the filtrates evaporated to syrupy consistency. The residues were then dissolved in water and each made up to 250 c.c. Measured portions of these solutions were used for the determination of total nitrogen, nitrogen as ammonia, and basic nitrogen precipitated by phosphotungstic acid, following the methods used by E. Schulze.* Following are the results obtained:

ANTIALBUMID (A).

Total nitrogen in the fluid	1.2745 grams.
Nitrogen in phosphotungstic acid precipitate . . .	0.2782 "
Nitrogen in the form of ammonia	0.0585 "
Basic nitrogen, organic	0.2197 "

17.2 per cent of total nitrogen in the form of bases precipitable by phosphotungstic acid.

HEMIALBUMOSES (G).

Total nitrogen in the fluid	1.3917 grams.
Nitrogen in phosphotungstic acid precipitate . . .	0.4687 "
Nitrogen in the form of ammonia	0.0805 "
Basic nitrogen, organic	0.3882 "

27.9 per cent of total nitrogen in the form of bases precipitable by phosphotungstic acid.

* Schulze, E., Zeitschr. f. physiol. Chem., 1898, **xxv**, p. 360.

HEMIPEPTONE (I).

Total nitrogen in the fluid	1.3770 grams.
Nitrogen in phosphotungstic acid precipitate . . .	0.5492 "
Nitrogen in the form of ammonia	0.2637 "
Basic nitrogen, organic	0.2855 "

20.7 per cent of total nitrogen in the form of bases precipitable by phosphotungstic acid.

From these results we see that antialbumid differs only quantitatively from the hemi bodies in yielding nitrogenous organic bases on decomposition with a mineral acid; a fact which affords additional evidence that the difference between the so-called hemi and anti bodies is not due to the lack of specific groups or radicles in the anti body, but rather to some difference in the arrangement of the groups or the state of combination by which the latter body is rendered resistant to the enzyme trypsin. Particularly noteworthy is the large proportion of nitrogen split off from hemipeptone in the form of ammonia, over nineteen per cent of the total nitrogen appearing in that form.

In order to ascertain how far the possible presence of these " hexone " bases in antipeptone may affect the physiological action of the latter substance, an experiment was tried with a dog of 2.5 kilos body-weight in which 25 c.c. of a solution containing 0.73 gram of the mixed bases (from the phosphotungstic acid precipitate) in the form of acetates were injected intravenously. The substance injected contained 15.5 per cent of nitrogen when dry. As a result it was found that the clotting time of the blood was not noticeably affected, while arterial pressure quickly fell from 135 mm. to 50 mm. Hg., but returned to the normal within three minutes.

ON ABSORPTION FROM THE PERITONEAL CAVITY.*

By LAFAYETTE B. MENDEL.

THERE is abundant experimental evidence to indicate that when an intravascular fluid is separated from an extravascular one of different composition, an interchange of substances will take place between them.   Given a separating membrane, *e. g.*, a capillary wall, sufficiently permeable to the substances in solution, the two fluids will manifest a tendency to become equalized in composition.   Thus substances originally dissolved in the extravascular fluid only may pass into the intravascular solution according to the established principles of osmosis.†   That substances foreign to the blood can in this way be absorbed into the circulation directly through the blood-vessels has long been known.‡   In investigating the absorption of dissolved substances with reference to the channels by which they enter the circulation from the connective tissue spaces, it has been a customary procedure to introduce readily detectable compounds into these spaces, and to watch for their appearance in the blood or lymph stream under various conditions.   The outcome of these researches has been to attribute to the blood-vessels an important rôle in absorption from connective tissue spaces, quite in accord with the requirements of physical diffusion ; although it has by no means been denied that the lymphatics also may be engaged in the transfer from the tissue cavities to the blood.

The problem of absorption from the pleural and peritoneal

cavities has been studied by Starling and Tubby * among others. They introduced a colored substance — indigo-carmine or methylene-blue — in solution into the cavities, and observed the reappearance of the pigment in the urine after ligation of the thoracic ducts, or after introducing cannulas and collecting the lymph before its entrance into the blood stream. In their experiments the urine was collected through a cannula tied into the ureter, " or in most cases into the bladder, either through an opening into the abdominal wall, or through an opening in the urethra, which was exposed by splitting the symphysis pubis." They observed in this way that " 5–20 minutes after the injection of the coloured fluid into the serous cavities the urine became tinged with blue when indigo-carmine was used, or green when methylene blue was the substance employed. The urine, on shaking with air, rapidly deepened in tint, until the colour was as intense as that of the fluid injected. After a further lapse of time, varying from ten minutes to four hours, the lymph flowing from the thoracic duct also became slightly tinged, but the colour never deepened, on shaking with air, beyond a very light blue or green. The flow of lymph was not increased " (p. 143). The investigators add : " It might be argued from these experiments that the absorption of the colouring matter from the pleural or peritoneal cavity took place by means of the blood-vessels and by means of the lymphatics, the former process however being the quicker of the two. It is very doubtful, however, whether the slight colouration of the lymph which was observed in these experiments is occasioned at all by lymphatic absorption. If methylene blue or indigo-carmine be injected into the blood stream, the lymph flowing from the thoracic duct within half a minute becomes coloured. Now in these cases the colouring matter must have been present in the blood in order to have been excreted by the kidneys, and the colour of the lymph may be caused by a passage of coloured lymph from the blood vessels, not by any direct absorption of the blue from the serous cavities " (p. 144). The data presented thus indicate

* Starling and Tubby, Journal of Physiology, 1894, xvi, p. 140.

a direct interchange between the fluid in the cavities and the blood in the vessels. Cohnstein,* likewise, has found these phenomena in direct accord with the principles of osmosis.

Comparable with the preceding observations, and indicating the immediate importance of the blood-vessels in absorption within the tissues, are experiments by I. Munk.† He severed the truncus lymphaticus colli, which conducts away the total lymph from the head in the rabbit, and removed the lymph coming from the cephalic end of the duct. When strychnine was thereupon injected under the skin of the head, no delay in the oncoming or intensity of tetanic symptoms was observed, although the blood-vessels formed the only channel of communication with the general circulation. Furthermore, no strychnine could be detected in the lymph collected from the head. Again, Wertheimer and Lepage ‡ have demonstrated the active participation of the blood-vessels of the liver in the absorption of substances from the bile. When a solution of indigo-carmine was allowed to flow into the ductus choledochus of a dog at a pressure of 30 cm., the urine collected from the ureter was found to be blue some minutes before the lymph flowing from the thoracic duct showed a trace of the pigment. The authors conclude that the lymphatics play a subordinate part, at most, in this process of absorption.§

In a recent paper Meltzer ‖ has reviewed the investigations of Starling and Tubby. Admitting that their experiments "if confirmed, would indeed prove the correctness of the blood-vessel theory of absorption," Meltzer has repeated them and has presented the protocols of a series of further experiments which lead him to quite different conclusions. The method of experimentation was essentially that employed by

---

* Cohnstein, Centralblatt für Physiologie, 1895, ix, p. 403.

† Munk, I., Archiv für Physiologie, 1895, p. 387.

‡ Wertheimer and Lepage, Archives de Physiologie, 1897, p. 373.

§ Page 374: "Ces quelques exemples suffisent pour prouver non seulement que les vaisseaux sanguins prennent une part active à la résorption du pigment bleu, mais encore que les lymphatiques n'y ont qu'une part très restreinte."

‖ Meltzer, Journal of Physiology, 1897, xxii, p. 198.

Starling and Tubby. Colored substances, or potassium fer-rocyanide, were introduced into the peritoneal cavity; the lymph was collected from the thoracic duct and the urine by means of a catheter introduced into the bladder through an external opening in the urethra. An extensive opening of the abdominal cavity was thus avoided. " In all of the ex-periments, the coloured fluid or characteristic Prussian blue appeared invariably in the lymph distinctly earlier than in the urine. . . . The lapse of time between the injection into the abdomen and the appearance in the lymph was in all the ex-periments approximately the same — an average of fifteen minutes. . . . On the other hand the time for the first ap-pearance of the colour, etc., in the urine varied considerably in the different experiments." Meltzer adds: " In the few experiments I made by injecting coloured fluid directly into the circulation, the colour appeared in the lymph before it appeared in the urine; the interval for the lymph was ap-proximately constant, while the interval for the urine was quite variable — between seven and thirty-four minutes." [*] In view of this reverse of the result obtained by Starling and Tubby, Meltzer has rejected the theory that the peritoneal absorption takes place through the walls of the blood-vessels and not by way of the lymphatics.

The preceding discussion has led Starling [†] to repeat his earlier experiments with due regard to the precautions em-phasized by Meltzer, and he has failed to discover any fallacy in them. He calls attention, however, to the necessity of emptying the bladder at regular intervals of two or three minutes by pressure on the abdominal wall, when the urine is collected through a cannula inserted directly into the bladder. Otherwise the urine is apt to accumulate in the latter and only the overflow is collected; a delay is thus occasioned between the secretion of a portion of urine and its appearance in the cannula. To this point I shall refer again somewhat later.

* Meltzer, Ibid., p. 203.
† Starling, Journal of Physiology, 1898, xxii, p. xxii.

In the course of some experiments on the action of certain lymphagogues * it occurred to me that if colored substances introduced into the abdominal cavity are carried into the circulation mainly through the lymphatics, an acceleration of the lymph flow might be accompanied by a more rapid or extensive transference of the pigment along this channel.† The first experiment was on a dog in which an intravenous injection of heteroalbumose had produced pronounced lymphagogic effects together with complete stoppage of urinary flow.   Ten c.c. of strong indigo-carmine solution were introduced into the peritoneal cavity.   No blue could be detected in the lymph collected within an hour; the bluish tint of a sample of blood serum, however, gave evidence of an active absorption.   In another animal previously receiving an intravenous injection of hemipeptone, the influence of which had already disappeared, fifteen c.c. of the blue solution were introduced into the peritoneal cavity.   The blue color appeared in the urine in *fourteen* minutes, in the lymph three minutes later.   Observations of this kind led to a series of experiments on normal dogs under a variety of conditions.   The technique of the experiments was essentially like that adopted by Starling and by Meltzer.   The dogs were anæsthetized (after 36 hours' fasting) by a subcutaneous injection of morphine sulphate followed by chloroform-ether administration. Cannulas were tied into the thoracic duct and both ureters. For collecting the urine the ureter method seems to me to be the preferable one, since it prevents any stagnation of fluid in the bladder.   But inasmuch as the method has been criticised because it occasions the opening of the abdominal cavity the urine was collected in some cases directly from the bladder, in bitches, by introducing a catheter exteriorly through the urethra.   The results obtained were not modi-

* Chittenden, Mendel, and Henderson, Amer. Jour. Physiol., 1899, ii, p. 162.

† In this connection it was, of course, remembered that the lymph formed through the influence of these "lymphagogues" has its *origin* primarily in the liver.

fied by this procedure, as the protocols will show. The colored fluid (indigo-carmine solution) was always introduced into the peritoneal cavity with a blunt pipette * through a small opening in the linea alba near the sternum. The abdominal wounds were all carefully stitched afterwards. The details of some experiments follow.

I. Dog, 16 kilos. Cannula in thoracic duct and in each ureter. The lymph flow in ten minutes was 2.3 c.c.; urinary flow was good. At 12.30, 20 c.c. strong indigo-carmine solution were introduced into the peritoneal cavity. At 12.46 the urine was tinged blue; the lymph continued normal in color until 12.58–12.59, when a faint blue tinge was evident. The urine, at this time, was perfectly dark with blue. At 1.05 the lymph showed a deeper blue, but the color was not intense; at 1.20 the color began to deepen, and still persisted when the experiment was concluded at 2.30. The urine continued to show a very dark blue color.

Thus the color appeared in the urine in *sixteen* minutes; in the lymph in *twenty-eight* minutes.

II. Dog, 20 kilos. Cannula in thoracic duct and in each ureter; cannula for bleeding in femoral artery. The urine and lymph flowed regularly, the latter at the rate of 1.9 c.c. in ten minutes. At 12.22, 20 c.c. saturated indigo-carmine solution were introduced into the peritoneal cavity. A bluish tint appeared in the urine at 12.31, becoming very deep at 12.34. The color began to show in the lymph at 12.45 and gradually increased in intensity. At 12.47 a blood sample was drawn; the serum was obtained by centrifugalization and was precipitated with alcohol. The alcoholic filtrate was decidedly blue at a time when the lymph was only beginning to show a trace of color. At 2.30, when the experiment was concluded, the urine was still intensely blue with pigment, while the lymph was light blue.

Thus the color appeared in the urine in *nine* minutes; in the lymph in *twenty-three* minutes.

---

* Adler and Meltzer caution against the use of a sharp cannula for this purpose, since it may enter the intestinal lumen occasionally: Journal of Experimental Medicine, 1896, i, p. 498.

III. Bitch, 9 kilos. Cannula in thoracic duct. Catheter introduced exteriorly through urethra into the bladder. Lymph flow in ten minutes was 1.9 c.c. The urine flowed readily whenever pressure was applied over the bladder; otherwise the flow was not regular, a few drops being expelled occasionally with respiratory movements. During the experiment the urine was expressed at intervals of about three minutes. At 4.01, 15 c.c. strong indigo-carmine solution were introduced into the peritoneal cavity. At 4.10 the urine was distinctly blue, and the color had become intense at 4.20, when the first bluish tint appeared in the lymph. The color deepened somewhat, but had decreased, if anything, at the conclusion of the experiment at 5.05. A post-mortem examination showed the blue color well distributed throughout the peritoneal cavity.

In this experiment the possibility of escape of color along wounds in the abdomen was excluded. Nevertheless, the color appeared in the urine in *nine* minutes; in the lymph in *nineteen* minutes.

IV. Bitch, 10 kilos. Cannula in thoracic duct; catheter in bladder as in Exp. III. Urine was readily obtained by gentle pressure. The lymph flow in ten minutes was 2 to 3 c.c. At 11.35 2 c.c. indigo-carmine solution were introduced into the peritoneal cavity. At 11.53 the urine showed the blue color, which soon deepened. No trace of blue could be detected in the lymph within an hour. Accordingly, at 12.43, 10 c.c. aqueous indigo-carmine solution were introduced in the peritoneal cavity. At 1.0 the urine was decidedly more blue than before; the lymph assumed a bluish tint (?) which became distinct at 1.05, growing deeper and remaining blue when the animal was bled to death at 2.15. Practically all the colored fluid had disappeared from the peritoneal cavity. The diaphragm alone was stained deep blue.

Thus the color appeared in the urine in *eighteen* minutes, while no blue could be detected in the lymph within an hour; and again in the urine *within seventeen* minutes, and in the lymph somewhat later. The experiment is interesting because of the method employed to collect the urine and of the *small amount* of indigo-carmine at first introduced.

The following details are included from additional experiments in which the lymph flow was accelerated by intravenous

injection of a lymphagogue previously to the introduction of the colored solution into the peritoneal cavity.

V. Dog, 20 kilos. Cannula in thoracic duct and in each ureter. Cannula for intravenous injection in facial vein. The flow of urine and lymph being found satisfactory, at 11.33–11.34 50 c.c. of 20 per cent sodium chloride solution were infused intravenously. In the succeeding twenty-five minutes 46 c.c. of lymph and 112 c.c. of urine were collected. At 11.44 12 c.c. strong indigo-carmine solution were introduced into the peritoneal cavity. Blue color appeared in the urine at 11.48, and rapidly increased in intensity. At 11.53 the lymph assumed a faint bluish tint, which grew more pronounced, but was never very deep. When the lymph flow diminished again, a solution of 5 grams of Grübler's " pepton " was infused intravenously at 12.15. The urinary flow ceased at once. The lymph continued blue in color, 26 c.c. being collected in the succeeding fifteen minutes.

Thus the color first appeared in the urine in *four* minutes; in the lymph in *nine* minutes.

VI. This experiment has already been referred to briefly. Dog, 15 kilos. Cannula in thoracic duct and in each ureter. A previous injection of heteroalbumose had stopped the urinary flow. The lymph flow in ten minutes was 4 c.c. At 5.0 10 c.c. indigo-carmine solution (in 0.7 per cent sodium chloride solution) were introduced into the peritoneal cavity. At 6.0 the serum obtained from a sample of blood showed a faint bluish tinge. No pigment could be detected in the lymph, which was still flowing steadily.

VII. Dog, 15.5 kilos. Cannula in thoracic duct and in each ureter. Cannula for intravenous injection in facial vein. After a solution of 8 grams of Grübler's " pepton" had been introduced into the latter, the urinary flow practically ceased. The lymph flow in ten minutes was 10.6 c.c. Immediately after the infusion of the albumose-peptone 10 c.c. saturated aqueous indigo-carmine solution were introduced into the peritoneal cavity. The lymph continued to flow abundantly; no blue could be detected in it within three hours. A post-mortem examination showed the pigment distributed throughout the abdomen; the thoracic cavity was entirely free from it.

A similar experiment was carried out with analogous results on a dog of 20 kilos. Nearly 50 c.c. of lymph were collected without the appearance of any pigment in it being noted.

The data presented agree closely with those already recorded by Starling. In every case the characteristic color appeared in the urine before it could be detected in the lymph flowing from the thoracic duct, the interval observed ordinarily being about ten or twelve minutes. The reverse results obtained by Meltzer with indigo-carmine solutions are briefly tabulated below for comparison.[*]

INDIGO–CARMINE EXPERIMENTS OF MELTZER.

Weight of Dog.	Amount of Fluid Introduced.	Time of First Appearance of Pigment in Lymph.	Urine.
kilos.	c.c.	min.	min.
28	50	8	26
..	500	11	23
18	50	12	40
10	50	13	23
30	50	13	44
22	76	14	80

In view of the criticisms of previous investigators, it may be well to point out that the tardy appearance of the pigment in the lymph has not been due to any retarded lymph flow in my experiments; the rate of flow varied ordinarily between 1.9 c.c. to 4.4 c.c. in ten minutes. The results are apparently independent of the size of the animal, and the data obtained by collecting the urine from the bladder agree in every respect with those furnished by the ureter method. The amount of colored solution introduced into the peritoneal cavity in my experiments has been relatively small compared with that used by Meltzer; this precaution has seemed desirable in view of the statement that " the absorption of such small amounts certainly afforded a greater similarity with that of the normal lymph than when the large quantities of fluids used by these other writers were employed."[†]   Thus, in their experiments on rabbits, Adler and Meltzer used 1 to 2 c.c. of fluid.

[*] Meltzer, Journal of Physiology, 1897, xxii, p. 198.

[†] Adler and Meltzer, Journal of Experimental Medicine, 1896, i, p. 484.

It is not difficult to understand the prolonged absence of pigment from the lymph in those cases where only small quantities are introduced into the peritoneal cavity. The foreign substances, being quickly transferred directly into the blood stream, are at once taken up and eliminated by the kidneys, — as the intravascular injection of pigment also indicates. In this way little material is left to pass into the lymphatic system from the lymph spaces by this relatively slower process, or by subsequent transference from the blood-vessels.

It has already been mentioned that Starling and Tubby express doubt regarding the direct absorption of coloring matter into the lymphatics in their experiments, and emphasize the possibility that the coloration of the lymph may be due to a "passage of coloured lymph from the blood vessels." Thus they observed that a colored substance introduced intravenously may appear in the lymph flowing from the thoracic duct within half a minute. So far as I am aware, no other comparable observations are recorded showing so brief an interval. For sodium salicylate, which does not influence the lymph flow, Tschirwinsky [*] has observed from four to seven minutes; Cohnstein [†] has found four or five minutes to intervene after potassium or sodium ferrocyanide solutions are injected, and for sodium iodide the writer [‡] has repeatedly observed similar intervals. A few additional data obtained with indigo-carmine are given here: —

VIII. Dog, 20 kilos. Cannula in thoracic duct and in each ureter; cannula for intravenous injection in right facial vein. Rate of flow of lymph in ten minutes was 2.3 c.c.; of urine was 1.1 c.c. At 11.59 to 12.0 10 c.c. of strong indigo-carmine solution (in 0.7 per cent sodium chloride solution) were infused into the vein. At 12.9 the blue color was distinct in the urine. At 2.45 there was still a good flow of urine and lymph; the latter had not yet shown a trace of blue, while the color of the urine had

[*] Tschirwinsky, Centralblatt für Physiologie, 1895, ix, p. 49.
[†] Cohnstein, Archiv f. d. ges. Physiol., 1895, lix, p. 509.
[‡] Mendel, Journal of Physiology, 1896, xix, p. 227.

gradually grown less deep, now showing a light green tint. Therefore at 2.45 a further 20 c.c. of the same indigo-carmine solution were infused into the vein. At 2.55 the urine grew deep blue a second time, and at 3.08 the lymph began to show a faint blue tinge which scarcely increased in color. Lymph flow in succeeding forty minutes was 10 c.c. The dog was killed by bleeding and the thoracic duct showed no blue color in any part of its course.

Thus the color appeared in the urine in *ten* minutes; in the lymph in *twenty-three* minutes.

IX. Dog, 11 kilos. Cannula in thoracic duct and in each ureter; cannula for intravenous injection in right facial vein. The dog had been without food or water for two days; 50 c.c. 0.7 per cent sodium chloride solution had been infused slowly into the facial vein. Lymph flow in ten minutes was 3.1 c.c.; urinary flow was 0.9 c.c. At 11.42–11.44 15 c.c. strong indigo-carmine solution were slowly introduced intravenously. At 11.59 the urine was colored blue and quickly grew intense in tint. At 12.05 the lymph showed a faint bluish tint, which grew slightly deeper; but at 12.25 the color was doubtful. Therefore 25 c.c. indigo-carmine solution were again injected, whereupon at 12.33 the lymph again showed the blue tint, which deepened somewhat to light blue.

Thus the color appeared in the urine in *seventeen* minutes; in the lymph in *twenty-three* minutes (or with a larger quantity of colored solution in *eight* minutes).

X. Dog, 15 kilos. Cannula in thoracic duct and in each ureter; cannula for intravenous infusion in left facial vein. Urine and lymph flow well; lymph in ten minutes was 3 c.c. At 12.20–12.21½ 20 c.c. 1 per cent indigo-carmine solution in 1 per cent sodium chloride solution were introduced into the vein. At 12.28½ the urine was deep blue; at 12.32 a trace of blue appeared in the lymph and gradually increased somewhat in depth of color. At 1.0 20 c.c. 20 per cent sodium chloride solution tinged with indigo-carmine were infused. The acceleration in the flow of urine and lymph immediately followed. The urine grew less deeply blue; the color was missed entirely in the lymph.

Thus the color appeared in the urine in *eight* minutes; in the lymph in *twelve* minutes.

In similar experiments Meltzer [*] has reported that the color appeared in the lymph before it appeared in the urine.   Detailed protocols of the experiments have not been published, and few definite statements are made with regard to the urinary flow in these observations, or in those already tabulated.   It is difficult to resist the conclusion that the differences between our results are perhaps attributable to variations in the method of collecting the urine.   Thus in Meltzer's experiments on direct intravenous injection of colored fluid, intervals of *seven* to *thirty-four* minutes elapsed before color was detected in the urine; with intraperitoneal injections intervals of *twenty-three* to *eighty* minutes are recorded.   The results were apparently independent of the size of the animal used.   The longest interval that I have observed was *eighteen* minutes in Exp. IV. when only 2 c.c. indigo-carmine solution were introduced.   Furthermore the actual amount of pigment appearing in the lymph was in no case equivalent to more than a very small fraction of the pigment introduced into the animal, or found in the urine.   A more satisfactory explanation does not suggest itself at present; there are, however, quite different and perhaps more satisfactory ways of arriving at an answer to the main problem concerned.[†]

The experiments recorded in this paper are thus in no way opposed to the " blood-vessel theory " of the absorption of foreign substances from the peritoneal cavity.   Rather they conform with the results which are demanded by the conditions and principles discussed at the outset.

[*] Meltzer, Journal of Physiology, 1897, xxii, p. 203.

[†] In a recent paper on the absorption of various intraperitoneal fluids when the lymph channels are excluded (in the rabbit), Róth concludes: "Dabei findet eine langsame Resorption der isotonischen Lösung durch die *Blutgefässe* statt." Archiv für Physiologie, 1898, p. 545. The older similar experiments by Hamburger have been criticised by Adler and Meltzer, Journal of Experimental Medicine, 1896, i, p. 482.

OBSERVATIONS ON THE NITROGENOUS METABOLISM OF THE CAT, ESPECIALLY ON THE EXCRETION OF URIC ACID AND ALLANTOIN.*

By LAFAYETTE B. MENDEL and ERNEST W. BROWN.

THE experiments which form a part of this paper were begun in continuation of a previous investigation in this laboratory on the excretion of kynurenic acid in various animals.† Our earlier experience had shown that kynurenic acid is absent from the urine of the cat during both fasting and proteid feeding, — conditions under which this acid is regularly found in the dog. The largely increased excretion of kynurenic acid found in the dog during the increased proteid metabolism caused by phlorhizin administration led us to try similar experiments on the cat. It was reasoned that if kynurenic acid were a normal product of metabolism which is ordinarily destroyed as soon as it is formed in this animal, then by bringing about an unusually marked stimulation of metabolism this product might appear, owing to the temporary inability of the organism to utilize all the intermediary substances formed. Extracts from protocols follow : —

Phlorhizin experiments. — I. A medium sized cat received subcutaneously in four doses 1.5 grams phlorhizin dissolved in dilute sodium carbonate solution. The urine of two days, including the day of injection — 208 c.c. — was examined for kynurenic acid by Capaldi's ‡ method with negative results.

II. A very large cat received subcutaneously a total of two grams of phlorhizin in two doses with an interval of eighteen hours. The greater portion of the urine of four days — 209 c.c.

—was examined for kynurenic acid with negative results. On the two days of largest sugar excretion, the urine contained 10.0 grams of dextrose and 3.92 grams of nitrogen.

These experiments failed to demonstrate any production of kynurenic acid in the cat. We have made additional unsuccessful attempts to find kynurenic acid in the urine of the same animal under conditions of fasting and during an abundant diet of both meat and pancreas. There is therefore no modification of our earlier conclusion to be suggested.[*]

EXPERIMENT I.

Four cats were used in this experiment. The meat fed was chopped lean beef; the thymus was a preparation (Armour's) of the desiccated glands, and was fed mixed with meat.

1899.	Vol. of Urine.	Uric Acid.	Food.
	c.c.	grm.	grm.
II. 21	450	——[†]	Meat, 450.
22	300	——[†]	" "
23	210	0.112	" " thymus, 25.
24	350	0.215	" " " 50.
25	334	0.291	" " " 100.
26	320	0.108	" 500.
27	190	0.015	" "
28	287	0.012	" "
III. 1	260	0.011	" "
2	170	0.009	None.

Experiments on uric acid excretion. — The negative results just recorded induced us to ascertain whether similar differences exist between the organism of the cat and the dog in respect to other important products of metabolism. Our attention has thereby been directed particularly to the excretion of uric acid and allantoin. Regarding the occurrence of uric acid in the urine of the cat few references are to be found in physiological literature. It is reported to be absent frequently from the urine of both cat and dog, having been found regularly only during animal diet and starvation. Dur-

---

[*] Cf. Mendel and Jackson, loc. cit., p. 27.

[†] Fæces in the urine; a few crystals of uric acid were separated.

ing a diet poor in proteids it has been observed to disappear.*
Since these observations were made before the introduction
of the newer methods for the complete precipitation of uric
acid, it may reasonably be questioned whether they possess
more than relative value.

EXPERIMENT II.

Two cats were employed in this experiment. The food consisted of lean
meat alone; meat mixed with desiccated thymus; fresh pancreas.

1899.	Vol. of Urine.	Uric Acid.	Food.
	c.c.	grm.	grm.
III. 10	170		Meat, 200.
11	130	daily average. 0.008	" "
12	117		" "
13	180	0.064	" " thymus, 25.
14	150	0.045	" " " "
15	195	0.092	" " " "
16	155	0.034	" "
17	150	0.005	" "
18			" 250.
19			" "
20	daily average. 112	0.009	" "
21			" "
22			" "
23			" "
24	170	0.011	Pancreas, ca. 200.†
25	120	0.014	None.†
26	270	0.159	Pancreas, 780.
27			Meat, 200–250.
28			" "
29	daily average. 88	0.005	" "
30			" "
31			" "

The method employed in the present experiments has
usually consisted in feeding several cats in the same cage
with various foods, collecting the urine and determining the
uric acid present by the Ludwig-Salkowski method.   In
several series the acid separated has been estimated by titra-
tion with permanganate solution instead of weighing.   No
attempt has been made to collect the urine for sharply defined

* See Huppert, Neubauer und Vogel's Analyse des Harns, 10te Auflage,
1898, p. 311.
† The cats rejected part of the food and refused to eat.

periods; the animals were allowed water ad libitum and they
discharged their urine with considerable regularity. The
collections were made daily, and it was found that the results
thus obtained with different diets during periods of several
days were sufficiently pronounced to afford definite conclu-
sions. Extracts from protocols will be found in Tables
I–VII.

EXPERIMENT III.

Two cats were employed. The diet consisted of the same foods as in the
preceding experiment. The pancreas fed was fresh, lean, sheep's pancreas.

1899.	Vol. of Urine.	Uric Acid.	Food.
	c.c.	grm.	grm.
III. 12			Meat, 200–250.
13	daily average. 86	0.014	" "
14			" "
15			" "
16	205	0.036	Pancreas, 310.
17	190	0.203	" 570.
18	180	0.115	" 411.
19	220	0.100	" 400.
20			Meat, 250.
21	daily average. 143	0.026	" "
22			" "
23	250	0.046	" ca. 200; thymus, 25.*
24	170	0.036	" little; " (?)
25	101	0.017	" very little (50).

The experiments recorded suffice to demonstrate the ready
production of uric acid after ingestion of foods rich in nuclein
substances, *e. g.*, thymus and pancreas. The persistence of
a high uric acid excretion frequently on the day following
the feeding of the glands is doubtless in part due to the
method employed in collecting the urine (lack of catheteriza-
tion) rather than to any characteristic after-effect of the diet.
The results show so complete an analogy with the similar
feeding experiments on man and on the dog,† that detailed

* Part of the food rejected on this and the following day.

† For references to the literature on thymus and pancreas feeding see:
(man) Huppert, loc. cit., p. 313; Th. Cohn, Zeitschrift für physiol. Chemie,
1898, xxv, p. 509; Hopkins and Hope, Journal of Physiology, 1898, xxiii, p.
271; Weiss, Zeitschrift für physiol. Chemie, 1899, xxvii, p. 216; Jerome

EXPERIMENT IV.

Three cats were employed. The diet consisted of lean meat, or fresh sheep's pancreas.

1899.	Vol. of Urine.	Uric Acid.	Food.
	c.c.	grm.	grm.
III. 31			Meat, 250.
IV. 1	daily average. 116	0.011	" "
2			" "
3			" "
4			" "
5	Lost.	——	Pancreas, 300.
6	335	0.083	" 500.
7	265	0.206	" 550.
8	210	0.160	" 550.
9	300	0.120	" 300.
10	daily average. 146		Meat, 250.
11		0.005	" "
12			" "

discussion seems unnecessary here. For the same reason feeding experiments with pure nucleins were omitted.

Experiments on allantoin excretion. — We have been unable to find any detailed references to the excretion of allantoin in the cat. Meissner * has reported finding allantoin in small quantities in the urine of several cats living on animal diet. It seemed especially desirable to investigate the possibility of allantoin excretion in the cat, since this substance has been detected only rarely in the urine of man, thus giving further evidence of noticeable differences between certain metabolic processes of the dog and some other animals closely related. Quite recently the pronounced excretion of allantoin in the dog has been noted by Minkowski † and by Th. Cohn ‡ after thymus feeding; similar observations after pancreas feeding

---

Journal of Physiology, 1899, xxv, p. 98; Taylor, American Journal of Medical Sciences, 1899, cxviii, p. 141; (dog) Minkowski, Archiv f. Exper. Pathol. u. Pharmakol., 1898, xli, p. 376.

* Meissner, Zeitschrift für rationelle Medicin, 1868, xxxi, p. 303.

† Minkowski, Centralblatt für innere Medicin, 1898, No. 19; Archiv f. Exper. Pathol. u. Pharmakol., 1898, xli, p. 376.

‡ Th. Cohn, Zeitschrift für physiol. Chemie, 1898, xxv, p. 507.

EXPERIMENT V.

Three cats were employed.   The pancreas fed was from calves.

1899.	Vol. of Urine.	Uric Acid.	Food.
	c.c.	grm.	grm.
V. 14	280		Meat, 500.
15	150	daily average. 0.029	" "
16	120		" "
17	190		" "
18	200	0.098	Pancreas, 500.
19	250	0.215	" 700.
20	350	0.156	Meat, 500.
21	300	0.137	" "
22	180	0.087	" 450.
23	180	0.032	" "

are reported by Salkowski.*   These results have since repeatedly been verified in this laboratory.

Our attention has been directed to the occurrence of allantoin under similar conditions of diet in the cat.   In no case has this substance been missed in the urine after thymus or pancreas feeding.   Thus, in one of the experiments, the urine obtained after feeding 175 grams of desiccated thymus (Armour's) to four cats was concentrated to a syrup and extracted with alcohol.   The alcoholic extracts deposited 1.6 grams of characteristic crystals on standing.   After recrystallization, the preparation began to melt at 215° C. and gave the characteristic reaction with furfurol.   An analysis of the preparation gave 34.99 per cent N; theory, 35.44 per cent N. The crystals were thus identified as allantoin.   Again, after feeding about $2\frac{1}{2}$ kilos of fresh pancreas, 2 grams of allantoin were separated in the form of characteristic crystals.   After recrystallization they began to melt at 216–217° C. and showed a nitrogen content of 35.12 per cent.   Salkowski † obtained 3.08 grams of allantoin from the urine of a small dog to which $1\frac{3}{4}$ kilos of ox-pancreas were fed in five days.   It is not uncommon to find crystals of allantoin separating from cat's

* Salkowski, Centralblatt für die Medicinischen Wissenschaften, 1898, p. 929.

† Salkowski, loc. cit., p. 930.

EXPERIMENT VI.

Three cats were fed. In addition to uric acid determinations, total nitrogen was estimated in the urine by the Kjeldahl method: and phosphoric acid by titration with uranium solution. The thymus was a desiccated preparation used in other experiments.

1899.	Volume of Urine.	Nitrogen.	P$_2$O$_5$.	Uric Acid.	Food.
	c.c.	grms.	grms.	grms.	grms.
IV. 30	120	} daily average 11.99	0.801	0.014	Meat, 300
V. 1	250				" 300
2	190				" 300
3	360	22.42	2.116	0.096	" 300; thymus, 100
4	380	22.99	2.300	0.519	" 300 " 100
5	250	21.27	1.326	0.050	" 300
6	150	18.86	1.056	0.015	" 300
7	} daily average 120	18.27	1.014	0.009	" 250
8					" 250

urine after pancreas feeding, even before the urine is concentrated. In view of Salkowski's experience with dogs,[*] we have searched for allantoin in the urine of the cat after meat diet, but without success.

Uric acid feeding. — Since it has been demonstrated that uric acid ingested, in the case of the dog, is excreted again in part as allantoin,[†] similar experiments were carried out on the cat. Thus, two cats used in previous experiments were fed 6 grams of uric acid mixed with 200 grams of chopped meat. The urine — 360 c. c. — collected on the three succeeding days was concentrated, and about 0.5 gram of allantoin was obtained. In another experiment, in which 4½ grams were fed with 200 grams of lean meat to two cats, 0.3 gram allantoin was obtained. In this respect also, then, the metabolic processes of the cat resemble those of the dog.

[*] Salkowski, Berichte der deutschen chemischen Gesellschaft, 1878, xi, p. 500.

[†] Salkowski, ibid., 1876, ix, p. 719.

Other Experiments.—Quinine is reported by various authors [*] to diminish the excretion of uric acid in man. We have tried one experiment to determine the influence of this alkaloid on uric acid and allantoin output in the cat after pancreas feeding.

EXPERIMENT VII.

Two cats were employed. The quinine sulphate was administered in small gelatin capsules.

1899.	Vol. of Urine.	Uric Acid.	Food.
	c.c.	grm.	grm.
VI. 1	143	0.004	None.
2	90	0.007	Pancreas, 250 ; Qi sulph. 1.5.
3	200	0.016	" 500; " 2.
4	180	0.027	Meat, 200.
5	340	0.030	" "
6	160	0.011	" "

It will be seen that the increase in uric acid output here observed falls considerably below that seen in other experiments (Exp's III, IV, V) with pancreas feeding. It is scarcely to be assumed that the effects observed are entirely due to deficient digestion and absorption of the material ingested. Allantoin was not detected in this urine after pancreas feeding ; this single experiment can, however, scarcely justify any far-reaching conclusions. The same statement applies to another experiment in which tannic acid was given in relatively large doses to cats, in view of the current statements regarding the excretion of allantoin in man under such conditions.[†] No allantoin was found in the urine, and the uric acid output was apparently not modified in any way.

We have tried to repeat the experiments of Borissow [‡] relative to allantoin excretion in dogs after subcutaneous injections of hydrazine sulphate. The symptoms observed in cats

---

[*] Cf. Thomas, Neubauer und Vogel's Analyse des Harns, 9te Auflage, 1890 p. 246 ; Bohland, Münchner medicinische Wochenschrift, 1899, xlvi, No. 16 ; Cushny, Pharmacology and Therapeutics, 1899, p. 352.

[†] Cf. Anschütz, v. Richter's Organische Chemie, 1897, i, p. 335 ; also Bohland, loc. cit.

[‡] Borissow, Zeitschrift für physiol. Chemie, 1894, xix, p. 499.

after administration of doses varying from 100 to 150 milligrams resembled in many respects those described for the dog by Borissow. No allantoin was detected; however, the number of our experiments is as yet too small to permit a definite conclusion.

Composition of Cats' Urine.— The lack of data regarding the normal composition of cats' urine has induced us to append the results of a few analyses carried out on urine obtained during various conditions of diet. The figures represent daily averages calculated from the total of several days. Several different animals are represented in the series. Total nitrogen was estimated by the Kjeldahl process; urea nitrogen by the Mörner-Sjöquist method; uric acid according to the Ludwig-Salkowski method; P_2O_5 by titration with uranium solution; SO_3 gravimetrically. Creatinine was found present in cats' urine and separated as the zinc chloride compound.

AVERAGE DAILY EXCRETION FOR THE CAT.

Diet.	Total N.	Urea N.	Uric Acid.	P_2O_5.	SO_3.
	grm.	grm.	grm.	grm.	grm.
Meat, ca. 100.	4.06	2.56	0.009	0.600	0.263
" 85.	4.55	2.67	0.007	0.400	. . .
" 85.	3.52	. . .	0.005	0.678	. . .
" 85.	3.38	2.42	0.004	0.694	0.242
" 85.	. . .	. . .	0.005	0.645	. . .
" 85.	. . .	. . .	0.008	. . .	. . .
None	. . .	. . .	0.003	0.610	. . .
"	. . .	. . .	0.003	. . .	. . .

SUMMARY.

1. Kynurenic acid is not excreted by the cat even during the increased proteid metabolism produced by phlorhizin administration.

2. The ingestion of thymus and pancreas tissue causes a marked increase in the uric acid output of the cat, thus corresponding with the similar observations on man and the dog.

3. Allantoin excretion is likewise observed after thymus and pancreas feeding, and after uric acid ingestion. Prelimin-

ary experiments have failed to demonstrate allantoin production after administration of hydrazine sulphate.

4. The ordinary daily uric acid output per kilo of body weight in the cat is scarcely smaller than that observed in man and the dog. Creatinine is ordinarily present in cats' urine in noticeable quantity.

OF THE OCCURRENCE OF IODINE IN THE THYMUS AND THYROID GLANDS.*

By LAFAYETTE B. MENDEL.

THE announcement of Baumann's discovery of iodine as a normal constituent of the thyroid gland directed attention to the possible occurrence of this element in other organs of the body. For the most part, the investigations in this direction have yielded negative results. In the case of the ovaries, the occurrence of iodine in very minute quantities has been reported by several investigators.[†] The presence of minimal quantities of iodine in the hypophysis has been asserted by some writers,[‡] while others,[§] have failed to detect it. In the adrenals,[||] spleen,[||] and salivary glands[¶] also, iodine has been detected. When the almost constant presence of iodine compounds in the thyroids of fully developed animals is considered, it cannot seem remarkable that iodine in some form should pass into the circulation and occasionally be found in various organs. In all the instances cited, however, the quantities actually found have been so small that renewed investigation is needed before any serious significance can be attached to existing statements. That iodine is by no means a constant constituent of some of these glands, my own experience has shown; thus I have examined relatively large quantities of

---

* Reprinted from the Amer. Jour. of Physiol., vol. iii.

† Barell, Chemisches Centralblatt, 1897, i, p. 608; Seyda, ibid., 1897; ii, p. 806; Lanz, Berliner klinische Wochenschrift, 1898, pp. 371–372.

‡ Schnitzler und Ewald, Chemisches Centralblatt, 1896, ii. p. 548; H. G. Wells, Journal American Medical Association, October–November, 1897. Reprint, p. 57.

§ Baumann, Münchner medicinische Wochenschrift, 1896, No. 14; v. Rositzky, Wiener klinische Wochenschrift, 1897, pp. 823–824.

|| Barell, loc. cit.

¶ Cunningham, Journal of Experimental Medicine, 1898, iii, p. 281 note.

ovarian substance (pig's) and salivary gland (ox's) without being able to detect this element.

In his earlier experience, Baumann [*] was unable to detect iodine in the thymus gland; later, however, in 200 grams of fresh thymus glands of the calf, he succeeded in detecting $\frac{1}{10}$—$\frac{1}{18}$ mgr. iodine.[†] Considering this result in connection with the observations of Mikulicz [‡] on the efficiency of thymus feeding in goitre, Baumann regarded it as probable that the thymus contains iodine in the form of thyroiodine (iodothyrin), although in much smaller quantity than in the case of the thyroid. Further observations on the thymus scarcely exist. Cunningham [§] has repeatedly tested the thymus for iodine without success, and suggests " that possibly accessory thyroid bodies were present in the thymus examined by Baumann." H. G. Wells analyzed the thymus glands of four children. " In one from a four months old child, and in one from a still-born infant, slight traces of iodine could be detected. In the other two, which were both from still-born infants, no traces whatever could be detected, even when three grams of the dried glands were examined at one time." [||]

The writer's attention was first directed more closely to this subject through the examination of a rather unusual accessory thyroid found in the thoracic cavity in a well defined case of acromegaly.[¶] In this case the thyroid proper, which was enlarged and weighed 101 grams, contained a relatively small amount of iodine, in correspondence with the histological findings on examination of the gland. Thus there was a marked increase in the connective tissue as compared with a normal gland; while the colloid material was decreased. The later observations of Hutchison [**] and Oswald [††] have shown, how-

* Baumann, Zeitschrift für physiol. Chemie, 1895, xxi, p. 825.
† Baumann, Münchner medicinische Wochenschrift, 1896, No. 14.
‡ Mikulicz, Berliner klinische Wochenschrift, 1895, No. 16.
§ Cunningham, Journal of Experimental Medicine, 1898, iii, p. 231 note.
|| Wells, H. G., loc. cit.
¶ Osborne, O. T., Transactions of the Association of American Physicians, 1897, p. 262.
** Hutchison, Journal of Physiology, 1896, xx, p. 474.
†† Oswald, Zeitschrift für physiol. Chemie, 1897, xxiii, p. 810.

ever, that the iodine content of the thyroid is particularly associated with the presence of colloid matter. The large accessory gland found in the median line high up in the thoracic cavity, just above the upper end of the sternum, weighed 36½ grams. From its location it was at first regarded as thymus. It showed a distinct "division into a cortical and medullary portion, the latter constituting somewhat more than half the diameter of the entire body, and being made up of fibrous tissue. The cortical portion resembles that of developing thyroid tissue as described by Halsted * as being found in dogs after partial extirpation of the thyroid gland." In the central portion no iodine could be detected; in the cortical portion, within the spaces of which colloid matter was observed, no less than six milligrams of iodine were found. It may be added that the patient had undergone no previous treatment involving the use of iodine compounds, which are well known to increase the iodine content of the thyroid.

The preceding details have been recorded not alone because of the unusual conditions observed, but particularly because they emphasize the care which becomes necessary in the removal of thymus tissue, to avoid contamination with possible accessory thyroids. In the parathyroids of the dog and rabbit Gley † has found a relatively greater content of iodine than in the thyroids of the same animals.

The data to be presented in this paper include analyses of both human and animal glands.‡ In searching for iodine, the finely-divided, dried gland-substance was fused in a nickel crucible with sodium hydrate and potassium nitrate, and this process — together with the succeeding colorimetric estimation of the iodine in the fusion products — was carried out according to the directions of Oswald. § Silver crucibles were never

---

* Halsted, Johns Hopkins Hospital Reports, i.

† Gley, Comptes rendus, cxxv, p. 312.

‡ Grateful acknowledgment is made to Professor H. B. Ferris of the Yale Medical School and to Dr. Joseph Roby of Rochester, who have furnished suitable material; and particularly to Armour and Company of Chicago, who have generously prepared various gland products for me.

§ Oswald, Zeitschrift für physiol. Chemie, 1897, xxiii, p. 275.

employed, since they may occasion loss of iodine owing to the formation of insoluble silver iodide. The reagents used were repeatedly examined for iodine with negative results. In testing the efficiency of the method when applied to large quantities of material, I have found no difficulty in recovering at least $\frac{1}{10}$ mgr. iodine when $\frac{2}{10}$ mgr. iodine as KI were added to about 15 grams of dried serum-proteids or fibrin.

A summary of a few analyses of thymus and thyroid glands from infants follow. The weight of each thymus varied from three-quarters to three grams, dried; the thyroids were somewhat smaller.

The thymus and thyroid glands of four infants at full term were examined for iodine with negative results. In the same glands from two infants about 24 days old, the thymus contained no iodine, while the thyroid contained 0.07 mgr. iodine. The thymus from the following individuals likewise showed no iodine whatsoever.

Infant	26	days	old;	cause	of death :	gastritis.
"	48	"	"	"	"	inanition.
"	2 months		"	"	"	ileo-colitis.
"	4½	"	"	"	"	pneumonia.
"	6	"	"	"	"	"
"	8	"	"	"	"	colitis.
"	20	"	"	"	"	ileo-colitis.

As regards the thyroid, these results correspond with the observations of Baumann [*] and of Miwa and Stölzner,[†] who likewise examined the thyroids of young infants without finding iodine in the great majority of cases. H. G. Wells,[‡] however, has reported the finding of traces of iodine in thyroids of infants at full term. With reference to the thymus it is evident that the absence of iodine is noticeable even in older individuals in which a considerable accumulation of iodine in the thyroid has already taken place. I have made similar observations in the case of a dog of ten kilos. The thyroids,

[*] Baumann, Zeitschrift für physiol. Chemie, 1896, xxii, p. 11.
[†] Miwa und Stöltzner, Jahrbuch für Kinderheilkunde, 1897, xlv, p. 87.
[‡] H. G. Wells, loc. cit., reprint, p. 25.

weighing 550 mgr. air dry, contained about 0.25 mgr. iodine, while no iodine could be detected in the large thymus weighing over two grams, air dry.

Although it may be questioned whether the minute traces of iodine found by Baumann on analysis of a very large quantity (200 grams) of thymus deserve the physiological significance frequently attributed to this single observation, I have repeated these experiments with large quantities of thymus. The fresh glands (calves') were dissected very carefully, and after treatment with absolute alcohol and ether were tested by the method already referred to. Thus in one experiment 213 grams of fresh thymus (yielding 39 grams dry substance) were examined with negative results; similar experiments were made with varying quantities of substance, but in no case could iodine be detected in this material. In two samples of desiccated thymus carefully prepared for me by Armour and Company, I have likewise been unable to find a trace of iodine, even when large quantities were fused; while I have not failed to detect very small quantities of iodine added to equally large amounts of the same desiccated material.

In two samples of commercial thymus preparations, however, I have detected traces of iodine. Nine grams of one of these, labelled "Desiccated thymus containing 50 per cent sugar of milk," showed a faint trace of iodine; another product "Desiccated thymus of the calf," repeatedly yielded about 0.07 mgr. iodine in twelve grams of the substance. In these cases the iodine found presumably was attributable to contaminating materials. Additional weight is lent to this view by the fact that of three commercial thymus preparations from the same source, only one showed traces of iodine; while one of the two iodine-free products had been prepared at my suggestion with particular care to avoid contamination with accessory thyroid bodies.

From the foregoing evidence it seems probable that iodine is not a normal constituent of the thymus and that Baumann's detection of that element in the gland was due to admixture of thyroidal tissue. Finally, the feeding experiment of

Baumann,[*] in which the thyroids of a small dog ingesting 26 pounds of thymus in sixteen days were found to contain 1.4 mgr. of iodine, loses the significance attributed to it, in view of the later experiments of Roos.[†] The latter investigator found that dogs frequently retain a considerable store of iodine in their thyroids even after six weeks' feeding with meat, — a diet free from iodine. The results of a single brief feeding experiment with thymus can therefore no longer be regarded as a confirmation of the existence of iodine in the gland fed.

The favorable results which have been reported for the thymus method of treatment in both true and exophthalmic goitre have been confirmed more recently through the further observations by Reinbach [‡] in Mikulicz's clinic. The success of thymus therapy indicates, as Reinbach points out, that the peculiar action of thyroid preparations in reducing goitre is nothing specific for the thyroid. Furthermore Cunningham § has demonstrated that thymus tissue yields substances equally as capable as thyroid extractives of palliating the acute cachexia in thyroidectomized dogs. That these substances do not contain iodine is pointed out by Cunningham and is particularly emphasized by my own experiments.

What light do these experiments throw upon the significance of the iodine content of the thyroid ? It seems to the writer that a variety of evidence leads to the conclusion early announced by Hutchison,[‖] that the physiological activity of thyroid preparations is due to substances associated with the iodine, rather than to that element itself. This, at least, applies to the ordinary action of thyroid feeding in goitre. Two reasons for this conclusion may briefly be stated. First, the thymus, which shows marked similarity to the thyroid in

* Baumann, Zeitschrift für physiol. Chemie, 1896, xxii, pp. 14–15.

† Roos, Zeitschrift für physiol. Chemie, 1899, xxviii, pp. 43–44.

‡ Reinbach, Abstract in Centralblatt für die medicinischen Wissenschaften, 1899, p. 276.

§ Cunningham, Journal of Experimental Medicine, 1898, iii, p. 225.

‖ Hutchison, Journal of Physiology, 1896, xx, p. 494; cf. also Reinbach, loc. cit.

its effect on goitre, contains no iodine. Second, the proportion of iodine in the colloid matter may be artificially increased to even ten times the normal amount without occasioning any increase in the activity of the preparation; * while artificially iodized proteids show relatively little action. In this connection the recent work of Roos † deserves attention. He found that the effects of equal quantities of dried thyroid in increasing the nitrogenous metabolism of dogs were apparently proportionate to the content of iodine in the glandular tissue fed. Equally varying was the efficacy of the different preparations in reducing the size of parenchymatous goitres. These facts, however, by no means compel the conclusion that the characteristic action is due to the iodine present; with equal probability we may assume corresponding variations in the accompanying active groups to which the iodine is perhaps attached merely as a factor of secondary importance. Under this interpretation of the facts known, it is not necessary to follow Roos ‡ in attributing the favorable action of thymus to the minute traces of iodine occasionally found in preparations of that gland.

SUMMARY.

1. The accessory thyroids in man may contain both relatively and absolutely more iodine than the thyroid proper of the same individual.

2. The observations that the thyroids of newly-born children contain no iodine are confirmed.

3. There is no satisfactory evidence to show that the carefully isolated thymus of man or animals contains iodine. Traces found by other observers were presumably due to adherent thyroidal tissue.

* Hutchison, Journal of Physiology, 1898, xxiii, p. 178.
† Roos, Zeitschrift für physiol. Chemie, 1899, xxviii, p. 40; cf. also Oswald, Ibid., 1899, xxvii, p. 40.
‡ Roos, Zeitschrift für physiol. Chemie, 1899, xxviii, pp. 50–51.

THE FORMATION OF MELANINS OR MELANIN-LIKE PIGMENTS FROM PROTEID SUBSTANCES.*

By R. H. CHITTENDEN and ALICE H. ALBRO.

THE brownish black animal pigments, collectively known as melanins, occurring normally and pathologically in the body, are characterized as a class by a somewhat high content of carbon, a relatively low content of nitrogen, and a variable, though usually high, percentage of sulphur. The presence of the latter element in very appreciable amounts constitutes one of the reasons for the belief that these substances have their origin in some proteid antecedent, while the absence of iron, in most cases, excludes the view that they originate from the blood pigment. In a recent contribution by Schmiedeberg †️ to the chemical composition and nature of melanins, emphasis is laid upon the possible origin of these substances in antialbumid. This view is seemingly based upon the partial resemblance in chemical composition between the latter substance and the melanins, — a resemblance which is indeed somewhat striking. As a cleavage product of the proteids, antialbumid is characterized by a comparatively high content of carbon and a low content of nitrogen, and Schmiedeberg concludes that melanin may result from antialbumid by a process of hydrolytic cleavage comparable to the method by which the latter body results from albumin itself. He further points out that the sulphur of the antialbumid may remain with the melanin, thus accounting for the large amount of this element usually found, while ammonia and water are split off according to the following equation : —
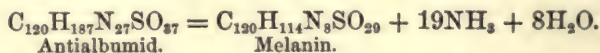
$$C_{120}H_{187}N_{27}SO_{37} = C_{120}H_{114}N_8SO_{20} + 19NH_3 + 8H_2O.$$
<div align="center">Antialbumid.   Melanin.</div>

This formula for antialbumid is based upon an analysis of antialbumid prepared by Kühne and Chittenden [*] from serum-albumin, while the formula for the melanin is based upon the analysis of a product (melanoidic acid) prepared by Schmiede-berg [†] by boiling serum-albumin with 25 per cent hydrochloric acid for twelve hours. Schmiedeberg likewise prepared a melanoidic acid from the fibrinoses contained in Witte's "pepton" by heating these proteoses with phosphoric acid for two months. Ascribing to the proteoses present in Witte's "pepton" a formula with 102 atoms of carbon, he formulates the production of the melanin as follows:

$$C_{102}H_{150}N_{30}SO_{31} + 18H_2O = C_{102}H_{98}N_{12}SO_{32} + 18NH_6 + 17H_2O.$$
Fibrinose.   Melanin.
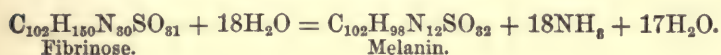
It is to be observed that these two artificial melanins or melanoidic acids differ from each other by nearly 6 per cent of carbon and 3 per cent of nitrogen, and that sulphur was determined in only one of the products, the amount found being 0.96 per cent. While the data may be considered as perhaps hardly sufficient to justify such definite expressions as the above formulæ, the general trend of the argument is exceedingly interesting and important. Any one who has worked much with the proteids is aware how readily these substances yield dark colored solutions on boiling with dilute mineral acids; a reaction not due to oxidation alone since it takes place readily even in the presence of reducing agents. It is thus evident that melanin-like substances may be formed from proteids by simple hydrolytic cleavage, and consequently the question at once arises how far the nature of the mother substance modifies the character of the resultant pigment. Further, is one proteid better adapted for the artificial pro-duction of a melanin than another proteid? How closely do the artificial melanins resemble in composition and general characters the natural pigments of this class? And lastly, how far can reactions of this kind be accepted as indicating

[*] Kühne and Chittenden, Zeitschrift für Biologie, 1883, **xix**, p. 176.
[†] Schmiedeberg, loc. cit., p. 66.

the mode of formation of the natural pigments common to the body in health and disease? Some of these questions we have endeavored to answer by the following experimental work.

Formation of Melanin from Antialbumid. — In view of the somewhat noticeable parallelism in composition between antialbumid and the melanins, attention was first directed to the possible formation of a pigment of this class by hydrolysis of antialbumid. For this purpose a pure antialbumid of known composition was necessary, and since the latter body is prone to slight alteration by too vigorous hydrolysis particular attention was paid to the conditions attending its formation. As the mother substance in the preparation of antialbumid, thoroughly washed coagulated egg-albumin was employed. In the hydrolysis, 2600 grams of the moist coagulum, pressed as dry as possible, were heated with 7 liters of 3.0 per cent sulphuric acid in a large flask, filled quite to the neck to diminish oxidation, at 100° C. (in a large Arnold sterilizer), for 10 hours. The gelatinous mass of impure antialbumid was thrown upon filters, allowed to drain, and after some washing with water, was transferred to a flask and heated with 3.5 per cent sulphuric acid for 10 hours, after which it was again filtered off and washed with water — by decantation and otherwise — until the washings were nearly or quite free from acid reaction. To remove any traces of unaltered proteid possibly present, as well as other impurities, the antialbumid was next warmed at 38° C. for 30 hours with an active solution of pepsin-hydrochloric acid (0.2 per cent HCl), the soluble products removed by filtration, and the residual antialbumid washed with water until the washings gave no reaction with the biuret test, nor with silver nitrate for chlorine. The washing was facilitated, after the bulk of the soluble matter had been removed, by carefully adding to the antialbumid suspended in water sufficient dilute solution of potassium hydroxide to make the mixture quite neutral to test paper, thus aiding the removal of any loosely

combined sulphuric acid. Finally it was washed with weak and strong alcohol and lastly with ether. When dry, the finely powdered substance was repeatedly boiled with distilled water to insure the complete removal of any adherent soluble salts.

Analysis of a sample of this product, dried at 110° C. until of constant weight, gave the following results:

1. 0.3397 gram substance gave 0.2098 gram H_2O = 6.86 per cent H and 0.6654 gram CO_2 = 53.45 per cent C.
II. 0.3439 gram substance gave 0.2114 gram H_2O = 6.83 per cent H and 0.6719 gram CO_2 = 53.29 per cent C.
III. 0.2259 gram substance gave by the Kjeldahl method 0.03054 gram N = 13.52 per cent N.
IV. 0.2502 gram substance gave 0.03429 gram N = 13.70 per cent N.
V. 0.6210 gram substance gave by fusion with NaOH* and KNO_3 0.1001 gram $BaSO_4$ = 2.21 per cent S.
VI. 1.0297 grams substance gave 0.0031 gram ash = 0.30 per cent.

PERCENTAGE COMPOSITION OF THE ASH-FREE ANTIALBUMID.

	I.	II.	III.	IV.	V.	Average.
C	53.59	53.45	. . .	. . .	. . .	53.52
H	6.86	6.83	. . .	. . .	. . .	6.84
N	. . .	. . .	13.56	13.74	. . .	13.65
S	. . .	. . .	. . .	. . .	2.22	2.22
O	. . .	. . .	. . .	. . .	. . .	23.77
						100.00

Comparison of the following figures shows the relationship in composition between the antialbumid and the mother substance from which it was derived.

	Coagulated Egg-albumin.†	Antialbumid.
C	52.18	53.52
H	6.93	6.84
N	15.81	13.65
S	1.87	2.22
O	23.21	23.77

Most conspicuous is the marked loss of nitrogen and the corresponding rise in carbon, — results which accord more or

* Pure NaOH prepared from metallic sodium and free from sulphur. The fusion was made over an alcohol lamp.

† Chittenden and Bolton, Studies in Physiological Chemistry, Yale University, 1887, ii, p. 130.

less closely with the earlier data obtained by Kühne and Chittenden.* Also noticeable is the somewhat larger percentage of sulphur present in antialbumid. A large amount of this sulphur exists loosely combined in the antialbumid molecule, and the question at once arises whether this loosely combined sulphur can be driven off by continued hydrolysis with dilute sulphuric acid. This point was tested by taking a portion of the antialbumid prepared as above and heating it further with 3 per cent sulphuric acid. After exposure at 100° C. in the sterilizer for 6 hours the acid fluid was found to yield a strong reaction for loosely combined sulphur, while the residue of antialbumid gave an equally marked reaction for loosely combined sulphur. The acid fluid was therefore filtered off, the antialbumid washed with water, and again heated for some hours with fresh 3 per cent sulphuric acid. This process was repeated until the aggregate period of heating had reached 38 hours, at the end of which time the last acid fluid was found free from loosely combined sulphur. The antialbumid remaining, however, still gave a striking reaction for sulphur with potassium hydroxide and plumbic acetate, thus showing that the loosely combined sulphur contained in the antialbumid molecule cannot be removed entirely by this method of hydrolysis. Especially noteworthy was the continued shrinkage of the antialbumid during this long process of heating with the dilute acid, until at the termination of the treatment the substance was greatly diminished in bulk, — a fact which accords with Schützenberger's † original observations that this substance, when heated continuously with dilute acid, slowly but progressively disappears. Somewhat noticeable, however, is the fact that such portion of the antialbumid as does resist this long continued action of the dilute sulphuric acid is not widely different in composition from the original antialbumid. Thus, analysis of the product remaining after the 38 hours' heating gave the following results:

* Kühne and Chittenden, Zeitschrift für Biologie, 1883, **xix**, p. 176.
† Schützenberger, Bulletin de la société chimique de Paris, 1875, **xxiii**, p. 161.

I. 0.1971 gram substance gave 0.1245 gram $H_2O = 7.01$ per cent H and 0.3937 gram $CO_2 = 54.48$ per cent C.

II. 0.3016 gram substance gave 0.6035 gram $CO_2 = 54.58$ per cent C.

III. 0.2972 gram substance gave by the Kjeldahl method 0.04113 gram N = 13.84 per cent N.

IV. 0.2093 gram substance gave 0.02860 gram N = 13.66 per cent N.

V. 0.5487 gram substance gave by fusion with NaOH and KNO_3 0.1058 gram $BaSO_4 = 2.63$ per cent S.

VI. 0.4770 gram substance gave 0.0015 gram ash = 0.31 per cent.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

	I.	II.	III.	IV.	V.	Average.
C	54.65	54.75	. . .	. . .	. . .	54.70
H	7.02	. . .	. . .	. . .	. . .	7.02
N	. . .	. . .	13.88	13.71	. . .	13.79
S	. . .	. . .	. . .	. . .	2.64	2.64
O	. . .	. . .	. . .	. . .	. . .	21.85
						100.00

From these data it is evident that the antialbumid which has resisted this long continued treatment with dilute sulphuric acid has gained somewhat in its content of sulphur and still more in its content of carbon. Nitrogen, on the contrary, remains essentially the same. This tendency of an antialbumid, on hydrolysis, to grow richer in carbon usually at the expense of the nitrogen is exceedingly characteristic. Thus, it was found by Kühne and Chittenden [*] on subjecting a sample of antialbumid (from egg-albumin) to the action of an alkaline solution of trypsin that the antialbumid remaining undigested contained 55.54 per cent of carbon when dry, while the original antialbumid contained 53.79 per cent of carbon. Similarly, an antialbumid, prepared from serum-albumin, containing 54.51 per cent of carbon and 14.31 per cent of nitrogen, on being subjected to the action of trypsin in an alkaline medium yielded an undigested residue containing 58.09 per cent of carbon and 12.61 per cent of nitrogen; results which clearly testify to the innate tendency of antialbumid under suitable conditions to undergo hydrolysis and presumably also cleavage with formation of a more insoluble and resistant body, of the antialbumid type, with a higher content of carbon.

[*] Kühne and Chittenden, loc. cit.

With a view to the possible preparation of a melanin, 30
grams of pure dry antialbumid (the first sample analyzed)
were placed in a flask with 300 c.c. of 10 per cent sulphuric
acid, the flask connected with an inverted Liebig's condenser,
and the mixture boiled directly over a flame for 79 hours.
When first heated, the acid was almost colorless, but as
decomposition progressed the color changed first to purple
and finally to jet black. Early in the course of the heating a
light film, shown later to be composed of fatty acids, was
deposited on the sides of the condenser, and the odor of
volatile fatty acids was very distinct.* As the boiling pro-
gressed, bright yellow crystals in rosettes were seen, both
within the condenser and clinging to the neck and sides of
the flask. These crystals were eventually collected and
tested. They burned with a blue flame, forming acid fumes;
they were insoluble in water, alcohol, and ether, but rapidly
soluble in carbon disulphide. From the latter fluid they
recrystallized in the characteristic rhombic octahedra of native
sulphur.

At the conclusion of the 79 hours of heating, and after the
acid fluid had cooled, the mixture was filtered through paper,
leaving a residue of black amorphous matter, while the clear
filtrate was quite black in color. Obviously, the fluid con-
tained considerable black pigment in solution, but owing to
the large admixture of leucin, tyrosin, and other substances
present, attempts to isolate the pure pigment from the solution
were not very successful. Attention was therefore directed
to the insoluble pigment on the filter paper. This was washed
with water until free from acid, and then with alcohol and
ether, in all of which it was insoluble. As the pigment was
found to be extremely soluble in weak alkalies, the precipitate
was treated with a very little 0.2 per cent potassium hydroxide
solution, in which it quickly dissolved, leaving a small slate-
colored residue (too small to identify) which was readily
washed free from the melanin-like substance. Although the
volume of the weak alkaline fluid with the washings now

* See R. Cohn, Zeitschr. f. physiol. Chem., 1896, xxii, p. 153.

amounted to full 500 c.c. the clear fluid was jet black in color and absolutely opaque to light. On neutralization of this fluid with dilute acetic acid the melanin was reprecipitated in large dark flocks, leaving a colorless transparent fluid. The pigment was filtered off, washed entirely free from salts with water, and thoroughly extracted with alcohol and ether, after which it was dried to a constant weight at 105° C. When dry the preparation weighed 1.498 grams, equal to practically 5 per cent of the original antialbumid.

On analysis this substance gave the following results:

I. 0.3598 gram substance gave 0.2249 gram $H_2O = 6.94$ per cent H and 0.7158 gram $CO_2 = 54.26$ per cent C.

II. 0.2044 gram substance gave by the Kjeldahl method 0.02453 gram N = 12.00 per cent N.

III. 0.2273 gram substance gave 0.02733 gram N = 12.00 per cent N.

IV. 0.3691 gram substance gave by fusion with NaOH and KNO_3 0.2063 gram $BaSO_4 = 7.70$ per cent S.

PERCENTAGE COMPOSITION OF THE MELANIN.[*]

	I.	II.	III.	IV.	Average.
C	54.26	. . .	. . .	. . .	54.26
H	6.94	. . .	. . .	. . .	6.94
N	. . .	12.00	12.00	. . .	12.00
S	. . .	. . .	. . .	7.7	7.70

A second decomposition, using a larger amount of the same antialbumid — 61 grams — was now attempted under exactly the same conditions as before, except that the acid mixture was boiled for 110 hours. Fatty acids and free sulphur were detected as before, while the same separation of a black pigment took place. The latter was collected on two filters and the precipitates washed as already described, after which the larger precipitate was dissolved in water containing a little ammonia, the clear filtered solution precipitated by neutralization with 0.2 per cent hydrochloric acid, and the precipitate thoroughly washed with water, alcohol and ether.

---

[*] The ash could not be determined with any accuracy owing to the scarcity of substance, but the small amount of ash left in the platinum tray in the determination of carbon made it quite clear that not more than a small fraction of 1 per cent could be present.

When dry, it weighed 2.728 grams. The smaller portion of crude melanin was dissolved in 0.2 per cent solution of potassium hydroxide and the clear filtered solution precipitated with dilute hydrochloric acid, etc., as just described. When dry this preparation weighed 0.675 gram. Hence, the total yield of melanin insoluble in the 10 per cent sulphuric acid amounted to 3.403 grams, or 5.5 per cent of the original antialbumid.

The melanin formed in this decomposition — the portion which had been purified by solution in dilute ammonia — gave on analysis the following results, after being dried at 105° C.

I. 0.3140 gram substance gave 0.2130 gram $H_2O = 7.53$ per cent H and 0.6631 gram $CO_2 = 57.60$ per cent C.

II. 0.2012 gram substance gave 0.1300 gram $H_2O = 7.17$ per cent H and 0.4267 gram $CO_2 = 57.85$ per cent C.

III. 0.2247 gram substance gave by the Kjeldahl method 0.02671 gram N = 11.88 per cent N.

IV. 0.2245 gram substance gave 0.02656 gram N = 11.83 per cent N.

V. 0.5479 gram substance gave by fusion with NaOH and KNO_3 0.1749 gram $BaSO_4 = 4.39$ per cent S.

VI. 0.4050 gram substance gave by fusion with NaOH and KNO_3 0.1258 gram $BaSO_4 = 4.27$ per cent S.

VII. 0.4616 gram substance gave 0.0025 gram ash = 0.54 per cent.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

	I.	II.	III.	IV.	V.	VI.	Average.
C	57.91	58.16	...	...	...	...	58.05
H	7.57	7.21	...	...	...	...	7.39
N	...	...	11.94	11.90	...	...	11.92
S	...	...	...	...	4.41	4.29	4.35
O	...	...	...	...	...	...	18.29
							100.00

The smaller fraction of this same melanin which had been purified by solution in 0.2 per cent potassium hydroxide contained the following percentages of nitrogen and sulphur:

0.2046 gram substance gave by the Kjeldahl method 0.02379 gram N = 11.63 per cent N.

0.4070 gram substance gave by fusion with NaOH and KNO_3 0.1272 gram $BaSO_4 = 4.29$ per cent S.

These results seemingly show that the sulphur and nitrogen content of the melanin is not materially affected by the character of the alkali used to dissolve it, — *i. e.* a fixed alkali, when dilute, causes no marked withdrawal of sulphur or nitrogen. Natural pigment from hair or epidermis when treated with 10 per cent solution of potassium hydroxide, and then reprecipitated by acid is liable to lose both nitrogen and sulphur,* but whether this is due to a change in the pigment itself produced by the strong alkali, or whether due to the withdrawal of contaminating substances derived from the original pigmentary granules, is uncertain.

Let us compare now the composition of our artificial melanins with that of the antialbumid from which they were derived.

	Antialbumid.	Melanin. 79 Hours' Boiling.	Melanin. 110 Hours' Boiling.	Antialbumid.†
C	53.52	54.26	58.05	54.70
H	6.84	6.94	7.39	7.02
N	13.65	12.00	11.92	13.79
S	2.22	7.70	4.35	2.64

The results seemingly justify the conclusion that these melanins formed from antialbumid originate not by simple hydrolysis, but by a process of hydrolytic cleavage, the pigment holding the position of a cleavage residue, the exact composition of which depends upon the extent or intensity of the cleavage process. Further, it is evident that this melanin-like residue, *i. e.* the true pigment, is either contaminated by some substance or substances, which accounts for the marked variation in composition, or else that under the term *melanins* we have a class of related bodies more or less alike in their physical properties but unlike in chemical composition. Certainly, our knowledge regarding the composition of the natural melanins lends favor to the latter view, for it is a well-known fact that certain melanins are exceedingly rich

* See Abel and Davis, Journal of Experimental Medicine, 1896, i, p. 391. Also, M. Nencki and N. Sieber, Archiv f. exper. Pathol. u. Pharmakol., 1887, xxiv, p. 17.

† After 38 hours' heating with 3 per cent sulphuric acid.

in sulphur (10 per cent), like the phymatorhusin of Berdez and Nencki,* while others, like the choroidal pigment,† are entirely free from sulphur.   Again, while the majority of these pigments contain practically no iron at all, others contain 0.5 per cent of this element.‡   Hence there may be justification for the suggestion made by Brandl and Pfeiffer § that the melanins should be divided into groups to be designated as ferro-melanins, sulpho-melanins, etc.   The melanins which we have prepared from antialbumid are practically free from iron, the ash containing only the merest trace of this element.   Somewhat suggestive is the fact that in both of our experiments with antialbumid, in spite of the difference in the length of the hydrolytic process, the yield of melanin in both cases amounted to about 5 per cent.

Formation of melanin from so-called hemipeptone. — Having demonstrated the possibility of preparing a melanin-like substance by the hydrolysis of antialbumid, the question naturally arose whether bodies of the so-called hemi class will likewise yield a melanin by hydrolysis.   To test this point, so-called hemipeptone formed by the hydrolysis of coagulated egg-albumin with 3 per cent sulphuric acid was employed.   The peptone was prepared by using the acid fluid, resulting in the formation of antialbumid.   The fluid was neutralized with ammonia, the filtered solution concentrated, and the albumoses separated collectively by saturation of the fluid with ammonium sulphate, after the method suggested by Kühne.‖   After complete removal of the albumoses, the excess of ammonium sulphate was separated by alternate concentration and crystallization, after which the last portions of the salt were removed by treatment with barium carbonate.¶   The filtrate,

---

* Berdez and M. Nencki, Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 346.

† Sieber, Ibid., 1886, xx, p. 362.

‡ See Brandl and Pfeiffer, Zeitschrift für Biologie, 1890, xxvi, p. 348.

§ Loc. cit.

‖ Kühne, Zeitschrift für Biologie, 1892, xxix, p. 1.

¶ See Chittenden, Mendel, and Henderson, Amer. Jour. Physiol. 1899, ii, p. 173.

freed from all traces of barium by cautious addition of dilute sulphuric acid, was concentrated to a syrup and the peptone precipitated by alcohol. The product was purified somewhat by repeated boiling with alcohol and thorough extraction with ether.

In the production of a melanin 90 grams of this hemipeptone were boiled for 98 hours with 2 litres of 10 per cent sulphuric acid. The fluid rapidly became black in color, and gradually there occurred a small separation of a black pigment. There was also noticeable, especially toward the end of the boiling, the odor of volatile fatty acids, and some slight crystallization of fatty acids upon the walls of the condenser could be detected likewise. There was however no evidence of the splitting off of free sulphur, so conspicuous with antialbumid. At the expiration of the 98 hours, the fluid was cooled, and the melanin-like substance collected by filtration. The very dark filtrate on standing and concentrating continued to deposit small portions of black pigment, the process being repeated until several fractions were obtained. The fluid, however, still gave evidence of the presence of considerable pigment in solution. The united fractions of pigment were washed thoroughly with water, then dissolved in 0.2 per cent solution of potassium hydroxide and the filtered fluid precipitated by neutralization with 0.2 per cent hydrochloric acid. The pigment was next washed free from soluble salts with water and extracted with alcohol and ether. When freshly precipitated, this melanin, unlike that prepared from antialbumid, was somewhat soluble in water, but if allowed to dry upon the filter it could be washed with water without loss. The amount of pigment obtained was 0.9 gram, equal to 1.0 per cent of the original hemipeptone.

When dried at 105° C. until of constant weight, it gave on analysis the following results:

I. 0.2435 gram substance gave 0.0815 gram H_2O = 3.72 per cent H and 0.5156 gram CO_2 = 57.75 per cent C.

II. 0.2068 gram substance gave by the Kjeldahl method 0.01986 gram N = 9.60 per cent N.

III. 0.2796 gram substance gave by fusion with NaOH and KNO$_3$ 0.0569 gram
   BaSO$_4$ = 2.79 per cent S.

IV. 0.2435 gram substance gave 0.0192 gram ash = 6.11 per cent.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

	I.	II.	III.	Average.
C	61.50	. . .	. . .	61.50
H	3.97	. . .	. . .	3.97
N	. . .	10.23	. . .	10.23
S	. . .	. . .	2.98	2.98
O	. . .	. . .	. . .	21.32
				100.00

It is thus plainly evident that the melanin obtained from hemipeptone is widely different in chemical composition from the corresponding body obtained from antialbumid. It resembles in its content of carbon the melanin-like body prepared by Schmiedeberg from Witte's "pepton," but differs widely from it in the amount of both nitrogen and sulphur. It would seem that the artificial melanins differ as widely among themselves in composition as the natural melanins obtainable from melanotic tumors, etc., or from normal pigmentary deposits. Certainly the contrast between the melanoidic acid with its 66 per cent of carbon obtained by Schmiedeberg from serumalbumin and the corresponding pigments with 54, 58, and 61 per cent of carbon respectively obtained by us from antialbumid and hemipeptone, is very striking.

Reactions of the melanins. — The pigments formed in the manner described above were practically insoluble in water, although, as stated, the sample obtained from hemipeptone was somewhat soluble when freshly precipitated. They were likewise insoluble in alcohol, ether, chloroform, etc., but readily soluble in exceedingly dilute alkaline fluids. They thus differ in some slight degree from the pigment isolated by Abel and Davis [*] from the negro's skin. Further, these pigments seemingly retain their solubility in dilute alkalies in greater degree than the pigments described by Abel and Davis or the melanoidic acid prepared by Schmiedeberg [†] from serum-albumin.

---

[*] Abel and Davis, Journal of Experimental Medicine, 1896, i, p. 386.

[†] Schmiedeberg, Archiv f. Exper. Pathol. u. Pharmakol., 1897, xxix, p. 66.

When dry, the pigments were more or less jet black in color, and when dissolved in dilute alkali they yielded yellowish brown, brown or black colored solutions according to the degree of concentration. These solutions show no absorption bands when examined before the spectroscope, but absorb a certain amount of light at the violet end of the spectrum. Unlike the melanin obtainable from the negro's skin,[*] our pigments were soluble in glacial acetic acid, but were not precipitable therefrom by potassium ferrocyanide. Alkaline solutions of these artificial melanins were completely bleached by chlorine.

Heated on platinum foil, our products, like the pigments described by Abel and Davis, gave off at first fumes of pyrrol, — tested by a pine-sliver moistened with hydrochloric acid, — but these soon ceased, leaving a coal-black residue very difficult of combustion.

From alkaline solutions, the pigments were precipitated by cupric sulphate, silver nitrate, plumbic acetate, and baryta water. By strong nitric acid the pigments were dissolved, but precipitated again on addition of water.

From these few reactions it is seen that our artificial melanins or melanoidins, to use Schmiedeberg's term, differ only in minor degree from the melanins or pigments obtained by Abel and Davis from the skin and hair of the negro, or from the melanins studied by other observers.

Comparison of the composition of the artificial melanins with that of natural melanins, etc. — Our own results bearing on the chemical composition of the melanin-like pigments obtainable by decomposition of proteids emphasize the view that these substances are many in number, and that, while having many points in common, they differ widely from each other in composition, owing no doubt in part to variations in the extent or intensity of the hydrolytic cleavage by which they are produced. This we fancy is a far more potent factor than the character of the individual proteid from which they are derived. Thus, in our experiments with antialbumid, the first prepara-

[*] Abel and Davis, loc. cit.

Origin of the Pigment.	Author.	Percentage Composition.				
		C	H	N	S	Fe
Hydrolysis of serum-albumin	Schmiedeberg*	66.27	5.49	5.57	...	...
Hydrolysis of Witte's "pepton"	Schmiedeberg*	60.34	4.86	8.09	0.96	...
Hydrolysis of Antialbumid†	Chittenden and Albro	54.26	6.94	12.00	7.70	...
Hydrolysis of Antialbumid‡	Chittenden and Albro	58.05	7.39	11.92	4.85	...
Hydrolysis of Hemipeptone§	Chittenden and Albro	61.50	3.97	10.28	2.98	...
Sarcoma (human) of liver and spleen	Berdez and Nencki ‖	53.58	4.22	10.59	10.13	...
Sarcoma of horse, liver and spleen	Berdez and Nencki ¶	58.52	8.92	10.48	2.78	...
Human urine, sarcoma	K. A. H. Mörner **	55.76	5.95	12.27	9.01	0.2
Tumor, horses' spleen	Miura ††	54.50	5.06	11.75	2.72	...
Human liver, sarcoma	Brandl and Pfeiffer ‡‡	53.58	4.00	9.91	3.02	0.53
Ox eyes, choroidal pigment	Sieber §§	59.90	4.61	10.81	0	0
Black and brown hair	Sieber §§	56.14	7.57	8.60	4.10	...
Epidermis of negro	Abel and Davis ‖‖	53.56	5.11	15.47	2.58	...
Hair of negro	Abel and Davis ‖‖	57.06	5.45	12.87	1.77	...

* Schmiedeberg, Archiv f. exper. Pathol. u. Pharmakol., 1897, xxxix, p. 67.
† 79 hours' boiling with sulphuric acid.
‡ 110 " " "
§ 98 " " "
‖ Berdez and M. Nencki, Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 346.
¶ Berdez and M. Nencki, Ibid., 1887, xxiv, p. 17.
** K. A. H. Mörner, Zeitschr. f. physiol. Chem., 1887, xi, p. 68.

†† Miura, Virchow's Archiv für Pathologische Anatomie, 1887, cvii, p. 250.
‡‡ Brandl and Pfeiffer, Zeitschrift für Biologie, 1890, xxvi, p. 348.
§§ N. Sieber, Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 362.
‖‖ Abel and Davis, Journal of Experimental Medicine, 1896, i, p. 392.

tion of melanin resulting from 79 hours' boiling of the proteid with sulphuric acid contained 54 per cent of carbon, while the second preparation resulting from 110 hours' boiling contained 58 per cent of carbon. Further, in the content of sulphur the differences were still more striking. The table on page 368, showing the composition of melanins from various sources, affords evidence of the extent to which these black pigments may differ from each other in chemical composition as well as indicating the extent to which the artificial melanins resemble the natural pigments.

These figures, which are fairly typical of the composition of the various melanins hitherto studied, show quite clearly how widely these bodies may vary in composition; yet throughout the entire list, as well as in many other preparations not tabulated, there is to be found almost invariably a relatively high content of carbon and a low content of nitrogen. Further, in all but the choroidal pigment, sulphur is very conspicuous. These facts, coupled with the inappreciable amounts of iron usually found, strengthen belief in the theory that these pigments, whether formed normally or as a result of pathological conditions, have their origin not in the hæmatin molecule but in proteid matter. Further, we see in the experiments of Schmiedeberg, as well as in our own results, evidence that serum-albumin, proteoses, antialbumid, and hemipeptone may all yield melanin-like substances by simple hydrolytic cleavage, — pigments which in composition and reaction differ from the natural pigments no more widely than the latter differ from each other. No two melanins are exactly alike in composition, and the artificial bodies, certainly, are exceedingly prone to vary in composition with variations in the method of preparation; especially, variations in the extent and intensity of the hydrolytic cleavage. In the hydrolytic cleavage induced by boiling acids, proteid substances tend to lose nitrogen, partially as ammonia and partially in the form of amido-acids and nitrogenous bases, while the artificial melanins simultaneously formed appear to have their origin in the carbon-rich residue left after the splitting off of these nitrogenous radicals. For this reason,

perhaps, antialbumid is especially well adapted to be the mother substance of a melanin.   Between antialbumid and the melanins there is a certain recognizable kinship in composition, which renders the formation of a melanin-like pigment from this peculiar form of proteid matter an easy task.   It is equally clear, from our experiments, however, that melanin may be formed likewise from such dissimilar proteid substances as so-called hemipeptone, from which we are forced to the conclusion that no one form of proteid matter is the sole antecedent of these peculiar brownish black pigments.

A NOTE ON THE CHOLESTERIN–ESTERS OF BIRDS' BLOOD.*

By ERNEST W. BROWN.

THE interesting investigations of Hürthle † have demonstrated that cholesterin occurs normally in combination with fatty acids in the blood-serum of mammals. It has been pointed out that the failure to recognize these cholesterin-esters has been due to the methods employed in the search for cholesterin. Thus the customary procedure has been to saponify ether extracts of animal tissues and fluids with alcoholic potash for the purpose of transforming any fats present into soaps, and in this way permitting a more successful subsequent separation of cholesterin by means of ether.‡ The method obviously precludes the possibility of obtaining cholesterin-esters as such. By avoiding the saponification, however, Hürthle has succeeded in demonstrating the presence of cholesteryl oleate in the blood-serum of the dog, sheep, pig, ox, and horse, and in the lymph obtained from the thoracic duct of the dog. Cholesteryl palmitate was also obtained, although in much smaller quantities; while cholesteryl stearate could not be isolated. The quantities of the cholesterin-esters present in the blood were approximately determined as follows:

	Cholesteryl Oleate.	Cholesteryl Palmitate.
Horse	0.08 per cent	0.006 per cent
Calf	0.09 "	0.008 "
Dog	0.12–0.22 "	

The percentage of cholesteryl oleate was observed to vary in the dog with the condition of the animal, being increased during hunger.§

* Reprinted from the Amer. Jour. of Physiol. vol. ii.

† Hürthle, Zeitschrift f. physiol. Chemie, 1896, xxi, p. 331.

‡ Cf. Hoppe-Seyler, Medicinisch-chemische Untersuchungen, 1866, p. 143.

§ Cf. Hoppe-Seyler, loc. cit., p. 145; Schulz, Fr. N., Arch. f. d. ges. Physiol., 1896, lxv, p. 299.

In his contributions to the chemistry of the blood, Hoppe-Seyler [*] has recorded analyses of the blood of the goose which indicate that cholesterin is present in the serum of this species in quantities approaching those of the ox.

In the course of some experiments in this laboratory, on the chemistry of birds' blood, the blood-serum of the hen, turkey, goose, and duck has been examined for cholesterin-esters. The observations made are recorded briefly here, since they verify and extend the investigations of Hürthle, whose methods have been employed, for the most part, in the preparation of the esters. The blood-serum was usually precipitated with three volumes of alcohol; after standing, the precipitate was filtered off and extracted with fresh alcohol for two or three days at 40° C. Good results were obtained by keeping the precipitate continually agitated in the warm alcohol by means of a slow current of air. The filtered extract deposited the characteristic small needle crystals of the oleate on standing in the cold, while the surface of the alcohol was usually covered with a slight film having a more or less crystalline character (cholesteryl palmitate). The film was separated as far as possible, and the larger mass of fine needle crystals, often grouped in rosettes, was filtered off, washed with cold alcohol, and dried in vacuo over sulphuric acid. The crystals thus obtained were weighed, in order to afford an approximate idea of the quantity of material obtainable from the various serums; this method, as Hürthle has observed, by no means gives accurate determinations, inasmuch as quite appreciable quantities of the corresponding ester remain in solution in the alcohol used. The precipitated serum residues were usually re-extracted with warm alcohol, and finally treated with alcohol-ether for the separation of the remaining cholesteryl palmitate. The yield of substance in the latter process was always small. The ester preparations obtained were purified by recrystallization from alcohol, until the melting points corresponded with those found by Hürthle. In some cases the composition of the product was further established by an

* Hoppe-Seyler, loc. cit., p. 145.

elementary analysis. The substances isolated all gave the cholesterin-like reactions described for them.

Hen serum. — Several preparations of cholesteryl oleate were separated from the serum of hens' blood and purified. They all melted at 43–44° C. An analysis of two products gave the following results:

ANALYSIS OF CHOLESTERYL OLEATE.

	Preparation I. per cent.	Preparation II. per cent.	Hürthle's Average. per cent.	$C_{44}H_{76}O_2$: Theory. per cent.
Carbon	82.68	82.49	82.84	83.02
Hydrogen	12.04	11.90	11.77	11.95

The approximate yield of cholesteryl oleate did not vary greatly. Thus:

   I. 500 c.c. serum yielded 0.24 gram = 0.05 per cent.
  II. 610 c.c.   "   0.19 " = 0.03 "
 III. 1660 c.c.   "   0.78 " = 0.05 "
 IV. 2200 c.c.   "   1.05 " = 0.05 "

From over four liters of serum about 0.2 gram substance was obtained, having a melting point (after recrystallization) of 77–78° C. and thus corresponding with the cholesteryl palmitate. The crystalline form also confirmed this deduction.

Turkey serum. — In one instance a relatively large yield of cholesteryl oleate was obtained from turkey serum. Thus:

  I. 600 c.c. serum yielded 0.37 gram = 0.06 per cent.
 II. 1000 c.c.   "   1.43 " = 0.14 "

The preparations melted at 43–44° C. and one of them (II.) showed the following composition on analysis:

    Carbon . . . . 82.84 per cent.
    Hydrogen . . . . 11.79 "

A preparation corresponding in crystalline form and solubilities with cholesteryl palmitate melted (after recrystallization) at 77–78° C.

Goose serum. — From 220 c.c. of this serum about 150 mgr. (0.07 per cent) of characteristic rosettes of needles were

obtained; they melted after recrystallization at 43–44° C., thus corresponding to cholesteryl oleate.

Duck serum. — Considerable difficulty was experienced in obtaining characteristic cholesteryl oleate preparations from duck serum. The products showed admixture of apparently amorphous material, and melted at about 60° C. From one half liter serum, however, about 0.4 gram (0.08 per cent) of crystals was obtained. After recrystallization, the crystals melted at 42–44° C. By extracting the serum residue with alcohol-ether, one half gram of a crystalline product was obtained. The preparation, when purified, melted at 76° C., giving evidence of the probable presence of a considerable quantity of cholesteryl palmitate (melting, when pure, at 77° C.).

The blood-corpuscles. — That cholesterin may occur in an uncombined state in the blood-corpuscles seems probable from the recent analyses of Abderhalden,[*] who found noticeable quantities of cholesterin in them, while fatty acids were absent, or present only in very small amounts. The older statements of Hoppe-Seyler [†] lead to similar conclusions; and Wooldridge [‡] stated that he obtained cholesterin free from fats and lecithin by extracting the stroma of blood-corpuscles with cold ether. In the present experiments, crystals of cholesterin were repeatedly obtained from the blood-corpuscles by direct extraction with ether. The corpuscles were separated from defibrinated blood by centrifugalization or by subsidence, and after treatment once or twice with one per cent sodium chloride solution to remove any adherent serum, were rendered laky and extracted. The ether extract corresponded in its behavior with the description given by Wooldridge; on evaporation it yielded a residue of needle-shaped crystals occasionally arranged in rosettes. After recrystallization

---

[*] Abderhalden, Zeitschrift f. physiol. Chemie, 1897, xxiii, p. 522; Ibid., 1898, xxv, p. 108.

[†] Hoppe-Seyler and Thierfelder, Handbuch der chemischen Analyse für Aerzte, p. 408.

[‡] Wooldridge, Archiv für Physiologie, 1881, p. 389.

from alcohol-ether the more characteristic rhombic tables appeared. These crystals showed the characteristic color reaction with chloroform and concentrated sulphuric acid, and the absence of fats or fatty acids was demonstrated by the fact that they melted at a temperature above 100° C. The corpuscles of the sheep, dog, hen, and turkey were examined, and cholesterin crystals obtained in every instance.*

I desire to acknowledge the kind advice of Professor Lafayette B. Mendel, at whose suggestion these experiments were carried out.

* Since the preceding account was sent for publication, a paper by E. Hepner, "Ueber den Cholestearingehalt der Blutkörperchen," has appeared in the Archiv f. d. ges. Physiol., 1899, lxxiii, p. 595. The cholesterin-content of the corpuscles of the dog and horse was determined ; free cholesterin was also detected in the blood-plasma.

METABOLISM IN THE SUBMAXILLARY GLAND DURING REST AND ACTIVITY.*

By YANDELL HENDERSON.

THE data at hand on the question whether, coincidently with the elimination of their characteristic products, the salivary glands take up nutriment during secretion, offer an apparent contradiction. Histological changes and the decrease both in weight and percentage of solids in an active gland indicate, as Heidenhain expresses it, that "at definite times the cells absorb from the blood or lymph definite substances; these substances are at definite times transformed; and at definite times these transformed substances are eliminated by the cells." † In contrast to this view are the experiments of Pawlow.‡ His investigation was performed upon dogs in which the chorda tympani and sympathetic nerve supplying the left submaxillary gland were severed. Secretion was excited in the right submaxillary by electrical stimulation of both sciatic nerves, for periods varying from one and a half to five hours. The saliva obtained, as well as the active and resting glands, were analyzed for nitrogen by the Kjeldahl method. Ten active glands from the right side yielded 1.872 grams of nitrogen, and the saliva secreted by them 0.416 gram — a total of 2.288 grams of nitrogen; while the ten resting glands from the left side gave 2.177 grams of nitrogen. Commenting upon these figures, Langley § points out that they "are not such as we should expect from the microscopical appearance of the gland cells. . . . The stimulated glands had lost during secretion about $\frac{1}{5}$ of their nitrogen-holding substance;" the saliva secreted contained approximately $\frac{4}{21}$ of the nitrogen of the resting glands,

* Reprinted from the Amer. Jour. of Physiol., vol. iii.

† Heidenhain, Hermann's Handbuch der Physiologie, v, p. 58.

‡ Pawlow, Centralblatt für Physiologie, 1888, ii, p. 137.

§ Langley, Schaefer's Textbook of Physiology, 1898, i, p. 488.

" so that presumably the active glands had taken up during secretion about 0.1 gram of nitrogen," or $\frac{1}{21}$ of the total amount. Thus, with regard to the nitrogenous or proteid metabolism of the active gland, anabolic and katabolic processes are to a considerable extent coincident.

The method employed by Pawlow is perhaps open to slight criticism, in that the stimulated glands were all on the right side; and apparently he did not exclude the possibility of errors due to exceptional variations between the glands on the two sides. Pawlow did indeed find the nitrogen content of the normal right and left submaxillary glands from ten dogs to be remarkably concordant, — amounting to 1.978 and 1.979 grams of nitrogen, respectively.* On the other hand, in eleven dogs Bidder found the left submaxillary heavier than the right; and Heidenhain reported a similar observation in two cases.

The question involved is of importance for the theory of secretion. It seemed desirable, therefore, to renew investigation with special reference to the criticisms outlined. The experience of the writer indicates that although differences do occur to the extent of 15 per cent in the weight of the glands on the two sides, yet the preponderance is not more often on the one side than the other. As a rule the normal right and left submaxillary glands agree closely in weight, dry solids, and nitrogen content, as the following typical analysis shows : —

TABLE I.

Large dog of fifteen kilos.

	Weight of Gland.	Dry Solids.	Nitrogen.
	grm.	grm.	grm.
Right submaxillary . .	8.48	2.136	0.2448
Left submaxillary. . .	8.24	2.125	0.2424

The method employed in the present investigation was as follows: Dogs of various weights were allowed to fast for

* Pawlow, loc. cit., p. 138.

twenty-four hours, in order to insure a resting condition of the glands. Water was given *ad libitum.* Anæsthesia was maintained (after the subcutaneous injection of a small dose of morphine) by continued administration through a tracheal cannula of a chloroform-ether mixture in just sufficient amount to insure the quiescence of the animal. Secretion was excited by electrical stimulation of the chordo-lingual nerve for periods varying from one and a half to seven hours, and the saliva was collected. The choice of the gland for stimulation was not confined to one side. In order to discover how far differences in the weight and composition of the glands can be assigned to merely vascular changes, the conditions of experiment were varied. Thus, in a few cases the chorda going to the resting gland was not disturbed; in the others, it was severed at the same time as the stimulated nerve. In two cases the animals were killed with chloroform, while a vigorous stimulation was maintained. In the others, the dogs were bled to death at periods which were varied from thirty seconds to fifteen minutes after the last stimulation. In all cases both glands were removed immediately after death, carefully separated from their capsules, and weighed. They were then comminuted, and treated with absolute alcohol for several hours; the alcohol was evaporated off; the tissue was dried at 110° for eight hours, and weighed. Finally, the saliva and the solids thus obtained were analyzed for nitrogen by the Kjeldahl method. As the results of the experiments failed to show any noticeable differences assignable to the variations in experimental condition, the differences between the stimulated and resting glands afford an indication and a measure of the changes within the secreting cells. Tables II and III give the results of nine experiments.

The figures show that during activity the submaxillary gland undergoes a marked loss of weight. This loss, calculated on the total weight of the nine active glands, as compared with the resting, amounts to 3 per cent. If Experiment III be excluded, it amounts to 7 per cent; and if Experiments VIII and II be considered separately, the difference in the

TABLE II.

Results of Analyses of Active and Resting Submaxillary Glands.

Number of Experiment.	Weight of Dog.	Stimulated Gland.	Duration of Stimulation.	Volume of Saliva.	Nitrogen in Saliva.	Weight of Glands.		Total Solids.		Nitrogen.	
						Active.	Resting.	Active.	Resting.	Active.	Resting.
	kilos.		hrs.	c.c.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
I	14	Left	3	17.0	0.0201	4.63	4.60	1.0436	1.1136	0.0994	0.1032
II	30	Left	2	32.0	0.0185	9.00	10.34	2.4232	2.5640	0.2940	0.2925
III	14	Left	1¾	17.0	0.0075	12.21	10.78	2.8930	2.6712	0.3525	0.3112
IV.	15	Left	4	48.0	0.0180	5.97	6.22	1.5263	1.5764	0.1740	0.1785
V.	12	Left	1¾	10.5	0.0120	3.99	3.90	...	...	0.1134	0.1088
VI.	10	Left	7	30.0	0.0120	3.88	3.58	0.7970	0.9135	0.0912	0.1020
VII.	8	Left	3	35.0	0.0140	2.97	3.32	0.7120	0.8790	0.0882	0.0972
VIII.	12	Right	6	100.0	0.0387	4.65	5.14	0.9905	1.2516	0.1212	0.1470
IX.	9	Right	1¾	8.0	0.0027	2.78	3.13	0.6470	0.7840	0.0738	0.0882
Totals	...	...	...	295.5	0.1485	49.58	51.01	11.0326	11.7528	1.4027	1.4286

TABLE III.

Expressing results of analyses in percentages.

Experiment.	Solids in Glands.		Nitrogen in Glands.		Nitrogen in Total Solids.	
	Active.	Resting.	Active.	Resting.	Active.	Resting.
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
I.	22.5	24.2	2.14	2.24	9.52	9.26
II.	26.9	24.8	3.27	2.82	12.13	11.40
III.	23.7	24.8	2.88	2.88	12.18	11.65
IV.	25.5	25.3	2.91	2.87	11.40	11.33
V.	. . .	. . .	2.84	2.66	. . .	. . .
VI.	23.5	25.5	2.69	2.84	11.44	11.16
VII.	24.0	26.4	2.80	2.92	11.69	11.05
VIII.	21.3	24.3	2.60	2.86	12.24	11.74
IX.	23.7	25.0	2.70	2.82	11.41	11.25
Average	23.9	25.0	2.76	2.77	11.50	11.10

weight of the active and that of the resting glands reaches
even 10 and 13 per cent, respectively.   The tables exhibit
also a diminution in the solids of the active glands which,
calculated on the totals for eight experiments, is equal to 6
per cent of the solids from the resting glands. If Experi-
ment III be excluded from the totals, the difference in content
of solids between the active and resting glands reaches 11
per cent; and in Experiment VIII it amounts to 20 per
cent. The figures in the first columns in Table III justify
the conclusion that in the secreting gland the content of solids
undergoes a relatively greater diminution than does the total
weight of the gland. The conclusions thus far reached are
therefore in accord with those of Heidenhain. On the other
hand, the nitrogen found in the active glands is only 1.4 per
cent less than in the resting glands. The nine active glands
contained 1.40 grams of nitrogen, the resting 1.42 grams, and
the saliva 0.14 gram. The averages for the middle columns
in Table III show identical figures for the *percentage* of nitro-
gen in the active and resting glands, and the experiments
taken separately exhibit nearly as close an agreement. These
observations, like those of Pawlow, indicate that the secreting

gland tends to replace its loss of proteid to a considerable degree during activity.

An explanation of the apparent discrepancy between the figures for the total solids and those for the nitrogen in the resting and active glands, seems to the writer to be found in the columns giving the percentage of nitrogen in the solids. In every experiment the solids from the active gland were relatively richer in nitrogen than those from the resting. This difference seems to justify the assumption that the active glands had become poorer in carbon, hydrogen, and perhaps oxygen. It suggests, also, that within the secreting cells there was a vigorous combustion of carbonaceous material, — carbohydrate, fat, the carbon moiety of proteid, or some such " explosive " combination of oxygen with substances rich in carbon as is held to be the source of heat and work in muscle. Indeed, our knowledge of secretion points to such a combustion as the source of the energy liberated within the gland. Although recent investigations [*] have not verified Ludwig's [†] observation that the saliva is warmer than the blood, it is generally held to be very probable that a liberation of heat occurs during secretion. The saliva contains a much larger amount of carbonic acid than does the blood.[‡] Physico-chemical considerations prove that the separation of a fluid like saliva, containing 0.4–0.6 per cent of salts, from a fluid like the serum of the blood, containing 0.7–0.8 per cent of inorganic matter, involves work. Ludwig showed that saliva is secreted at a pressure higher than that of the blood in the carotid; and O. F. F. Grünbaum [§] has recently published observations on the two kinds of work performed by the secreting glands. The microscopical changes observed in the secreting cells do not seem to offer any contradiction to the view that a combustion occurs. Observations of Heidenhain [‖] tend to show, also, that stimulation of the cervical

[*] Bayliss and Hill, Journal of Physiology, 1894, xvi, p. 351.

[†] Ludwig, Wiener medicinische Wochenschrift, 1860, pp. 433, 449.

[‡] Pflüger, Archiv f. d. ges. Physiol., 1868, i, p. 686.

[§] Grünbaum, O. F. F., Journal of Physiology, 1898, xxii, p. 385.

[‖] Heidenhain, Studien des physiologischen Instituts zu Breslau, 1868, iv, p. 66.

sympathetic and the resulting secretion of a saliva of small volume but large content of organic matter cause a loss of solids in the gland which is considerably less marked than that resulting from the abundant secretion excited by stimulation of the chorda tympani alone. Finally, the view that in the submaxillary gland proteid metabolism is more or less distinct from the processes of combustion and liberation of energy, accords both with the accepted theory of nutrition in the organism as a whole, and with the hypothesis of Heidenhain * that the elimination of water and salts and the elimination of the organic constituents of saliva are controlled by different mechanisms. For, on the one hand, in the proteid metabolism of the cells, controlled by the trophic nerve fibers, anabolism occurs to a certain extent coincidently with katabolism. The secreting gland tends to remain in nitrogenous equilibrium. On the other hand, the processes controlled by the secretory fibers are apparently performed at the expense of a combustion of carbonaceous material stored within the cells during rest, to become the source of heat and secretory work.

In conclusion, the writer desires to acknowledge his obligation to Professor Lafayette B. Mendel, both for the suggestion of the subject of this investigation, and for valuable criticism.

* Heidenhain, Hermann's Handbuch der Physiologie, v, pp. 50–51.

ON URIC ACID FORMATION AFTER SPLENECTOMY.*

By LAFAYETTE B. MENDEL and HOLMES C. JACKSON.

INTRODUCTORY.

IT is to-day a well accepted fact that uric acid formation occurs in mammalia under conditions quite different from those pertaining in birds and reptiles. The liver, which plays so important a rôle in the uric acid synthesis of the latter animals, is not the organ of chief importance for the similar process in the mammal. The experimental evidence upon which this position is based is diverse. Thus after exclusion of the liver from the circulation by means of Eck's fistula and ligation of the hepatic artery there is, if anything, an increase in the output of uric acid.† Again, in cirrhosis and hypertrophy of the liver, as well as in artificial degeneration of hepatic tissue,‡ no marked decrease in uric acid excretion has been noted. Such results would scarcely be expected if the liver were actively concerned in uric acid synthesis. In searching for an organ to which the production of uric acid might be delegated, many physiologists have turned their attention to the spleen. Thus Neumeister states: "The spleen stands in close relationship to uric acid formation, as is evident from experiments on animals and from pathological observations. This function is simply explained by the richness of the spleen in leucocytes, and therefore also in cell

† Hahn and Nencki, Archives des sciences biologiques de St. Pétersbourg, 1892, i. p. 401; De Filippi, Archives italiennes de biologie, 1899, xxxi, p. 211.

‡ Lieblein, Archiv für experimentelle Pathologie und Pharmakologie, 1894, xxxiii, p. 318.

nucleins, from the decomposition products — the nuclein bases — of which uric acid seems to arise, at least in the mammalia."[*] Hammarsten writes: "An increase in the quantity of uric acid eliminated has been observed by many investigators in lineal leucæmia, while the reverse has been observed under the influence of quinine in large doses, which produces an enlargement of the spleen. We have here a rather positive proof that there is a close relationship between the spleen and the formation of uric acid." Again, "a direct relationship between the spleen and the formation of uric acid in man, has been sought by several investigators. According to the investigations of Horbaczewski this relationship seems to be of an indirect kind, as it probably stands in close connection with the importance of the spleen to the formation of the leucocytes."[†]

These statements, to which those of other writers [‡] might be added, are for the most part due to the influence which the well-known investigations of Horbaczewski have exerted. This investigator observed that when spleen pulp is exposed to incipient putrefaction, considerable quantities of xanthin and hypoxanthin can be isolated from the mixture. If, however, the pulp at this stage is subjected to conditions which facilitate oxidation, e. g., shaking with air in the presence of blood, xanthin bases are no longer obtained, but uric acid is found in place of them. These observations have repeatedly been verified. Spitzer,[§] in particular, has studied the conditions under which this mode of formation of uric acid may be facilitated. His investigations lead to the conclusion that extracts of both spleen and liver, as well as the tissue pulp, may yield uric acid when subjected to a current of air for

[*] Neumeister, Lehrbuch der physiologischen Chemie, 1897, p. 512.

[†] Hammarsten, Textbook of Physiological Chemistry, translated by Mandel, 1900, pp. 200, 431.

[‡] E. g., Stadthagen, Archiv für pathologische Anatomie, 1887, cix, p. 403; Bunge, Lehrbuch der physiologischen und pathologischen Chemie, 1894, p. 314; Howell, American Textbook of Physiology, 1896, p. 273; Simon, Manual of Clinical Diagnosis, 1897, p. 349; Schreiber, Ueber die Harnsäure unter physiologischen und pathologischen Bedingungen, Stuttgart, 1899, p. 90.

[§] Spitzer, Archiv für die gesammte Physiologie, 1899, lxxvi, p. 192.

some time, even when putrefaction is absolutely excluded. This reaction was found to be characteristic for the two organs mentioned, namely, the liver and spleen, and could not be obtained with the kidney, pancreas, thymus, or blood. Furthermore, Spitzer ascertained that pure hypoxanthin and xanthin are transformed into uric acid by the oxygen of the air, when they are dissolved in extracts of the liver and spleen. Adenin and guanin may apparently undergo the same transformation, though to a far smaller extent. Spitzer concludes that the peculiar behavior of the spleen and liver, as contrasted with the other organs enumerated, may be interpreted to indicate that "even during life these two organs are the principal seats of uric acid formation." The author adds, however: "it must be remembered that observations made on dead material cannot be assigned to the living cells without reserve. We can therefore by no means deny the power of the last mentioned organs (pancreas, kidney, thymus, etc.) to form uric acid during life, although they perhaps possess the capacity in slighter degree than the liver and spleen." [*]

We have studied the importance of the spleen for uric acid formation in the living organism by a more direct method, namely, experiments on splenectomized animals. At the time that our investigation was begun we were unaware of the observation of Lo Monaco [†] on uric acid excretion in a man after extirpation of the spleen. He found the output after the operation approximately normal on a mixed diet; in any case, it was not noticeably diminished. The recorded observations on uric acid formation in hypertrophy and other abnormal conditions of the spleen are uncertain and in part contradictory.[‡] They thus afford no definite answer to the problem.

[*] Spitzer, loc. cit., p. 200.

[†] Lo Monaco, Bulletino della società Lancisiani degli ospedali di Roma, 1894, xiv, p. 102 ; also Schmidt's Jahrbücher, 1896, cclii, p. 109.

[‡] Cf. for example, Stadthagen, Archiv für pathologische Anatomie, 1887, cix, p. 390 ; Thomas, Neubauer and Vogel's Analyse des Harns, 1890, p. 241 ; Simon, Manual of clinical diagnosis, 1897, p. 349. Schreiber, loc. cit., 1899, p. 91.

EXPERIMENTAL.

The animals used were the dog and the cat. In removing the spleen the suggestions of Laudenbach [*] were usually followed, and the animals all made a very rapid recovery from the operation. Since the character of the diet is now recognized to be of fundamental importance in uric acid production, our feeding experiments were primarily directed towards ascertaining the influence of those foods which are known to be uric acid precursors, namely, the nucleins. *No diminution in uric acid production was observed in any case after splenectomy.* The uric acid output was observed during hunger and on a diet of casein, and subsequently the influence of uric acid forming food was noted. For this purpose we fed sheep's pancreas, which experience in this laboratory has demonstrated to occasion marked uric acid excretion. The characteristic excretion of allantoin first noted after pancreas feeding by Salkowski [†] in the dog, and by one of us in the case of the cat,[‡] was likewise always observed. Thus, from the urine of a spleenless dog to which $1\frac{1}{4}$ kilos of fresh sheep's pancreas were fed in three days, no less than 0.85 gram of allantoin crystallized out on concentration. A small spleenless cat fed with one kilo of fresh sheep's pancreas in five days, yielded 0.65 gram of allantoin in a similar manner. We have had occasion to feed lymphatic glands, such as are frequently found abundant throughout the pancreatic tissue of sheep and in the submaxillary region of the ox. In each case a large rise in the uric acid output was noted in both normal and spleenless animals; the yield of allantoin was noticeably large. So far as we are aware, these observations are new and give further indication of the importance of glandular tissue of this type in uric acid production.

Protocols of the feeding experiments with splenectomized

---

[*] Laudenbach, Archives de Physiologie, 1896, p. 698.

[†] Salkowski, Centralblatt für die medicinische Wissenschaften, 1898, p. 929.

[‡] Mendel and Brown, Amer. Jour. Physiol., 1900, iii, p. 261.

animals are given below; the uric acid was determined by the Ludwig-Salkowski method.

TABLE I.

Medium sized dog. The casein fed was freshly precipitated from skimmed milk and squeezed as dry as possible. The pancreas was obtained from sheep, and was freed from fat and sterilized. Water was freely allowed. The urine was not removed by catheterization, hence daily averages from three-day periods are given.

Day.	Food.	Urine.	
		Vol.	Uric Acid.
	grm.	c.c.	mgr.
1	Casein, 200	110	26 ⎫ (Average)
2	Casein.	145	26 ⎬ 25
3	Casein.	120	23 ⎭
4	Pancreas, 375	185	115 ⎫
5	Pancreas, 400	135	78 ⎬ 76
6	Pancreas, 300	75	36 ⎭
7	None.	145	105 ⎫
8	None.	55	⎬ 22 ⎬ 49
9	None.	175	⎭

TABLE II.

Medium sized dog. Pancreas prepared as in preceding experiment. Water ad libitum.

Day.	Food.	Urine.	
		Vol.	Uric Acid.
	grm.	c.c.	mgr.
1	None.	54	15 ⎫ (Average)
2	None.	50	15 ⎬ 14
3	None.	52	12 ⎭
4	Pancreas, 345	225	125 ⎫
5	Pancreas, 360	170	96 ⎬ 119
6	Pancreas, 530	130	188 ⎭

TABLE III.

Cat. The animal had previously been fed on casein for several days. The pancreas was prepared as above.

Day.	Food.	Urine.	
		Vol.	Uric Acid.
	grm.	c.c.	mgr.
1	Pancreas, 65	25	
2	Pancreas, 120	50	37
3	Pancreas, 100	65	
4	None.	35	
5	None.	20	2
6	None.	18	

The following protocol is added to demonstrate uric acid excretion during hepatic degeneration in a spleenless dog. The results are of interest because they illustrate the production of uric acid after exclusion of the function of the two

TABLE IV.

Spleenless dog.   Phosphorous poisoning.

Day.	Urine.			Remarks.
	Vol.	Uric Acid.	Total N.	
	c.c.	mgr.	grm.	
1	245	77	8.54	No food.
2	350	103	7.31	" "
3	290	163	9.01	" " ½ c.c. oleum phosphoratum, subcutaneously.
4	140	118	7.06	" " *
5	180	120	7.58	" " *
6	163	100	6.78	" " *
7	158	126	6.12	150 grams **pancreas** fed.
8	260	171	9.06	50 grams desiccated **thymus** fed. †
9	184	106	6.91	25 " " " "
10	148	52	3.83	25 " " " "
11	88	37	1.60	No food. Dog very weak.
12	. . .	. . .	. . .	Dog died.

* Uric acid was fed in capsules on these days for another purpose; most of it was again recovered in the fæces, and the absence of any corresponding rise in nitrogen output indicates that it was not absorbed.

† The dog vomited some of this food.

chief organs to which this process has been attributed. The action on the liver was brought about by subcutaneous injection of oleum phosphoratum. The presence of much bile pigment in the urine as well as the characteristic metabolic changes before death gave evidence of the hepatic action of the poison. Histological examination of the liver cells also revealed pathological changes in that organ. The dog had already received four injections of phosphorus oil during the twelve days preceding those here recorded.

CONCLUSION.

Our experiments demonstrate that the spleen is by no means the chief organ involved in uric acid production in the living body, if indeed it normally plays any part whatever in this process. After the exclusion of the liver and the spleen it is natural to turn to other forms of lymphoid tissue, and the lymphatic glands are at once suggested. It might be supposed that after splenectomy these glands take up the work of the spleen. Enlargement of the lymphatic glands has been recorded after removal of the spleen in man. But the very recent investigations of Vincent,[*] made to ascertain this point in the dog, fail to bring to light any permanent hypertrophy of the lymphatic glands after splenectomy. It seems improbable, therefore, that the formation of uric acid in the mammalia can be assigned at present to any definite organ, or groups of organs.

[*] Vincent, Journal of Physiology, 1900, xxv, p. ii.

THE AMIDE NITROGEN OF PROTEIDS.[*]

By YANDELL HENDERSON.

THE fact that, on decomposition with strong hydrochloric acid, a part of the nitrogen of the proteid molecule is obtained as ammonia was first revealed by Hlasiwetz and Habermann [†] in their now classic investigations on the cleavage products of the proteids. Following them, Nasse [‡] carried out a series of quantitative determinations as to the percentage of the nitrogen thus separated, and found marked differences between the different albuminous bodies in their content of this "loosely combined nitrogen." More recently these investigations have been repeated by Hausmann,[§] and also considerably widened in scope by quantitative analyses, not only of this "amide" nitrogen, but also of the "diamino" and "monoamino" nitrogen [||] obtained by decomposing proteids with strong hydrochloric acid.

The results so obtained have led Hausmann to regard the

[*] These investigations were first published under the title, Zur Kenntniss des durch Säuren abspaltbaren Stickstoffes der Eiweisskörper, in Zeitschr. f. physiolog. Chemie, Band xxix, Seite 47.

The experiments here recorded were completed before the publication of Hausmann's work. More recently Hausmann has published a second paper in support of his views, and Kutscher has put forward a criticism of them supported by considerable experimental data. These articles are: W. Hausmann, Zeitschr. f. physiolog. Chemie, Band xxix, p. 136 ; Fr. Kutscher, Zeitschr. f. physiolog. Chemie, Band xxxi, p. 215.

[†] Hlasiwetz und Habermann, Ueber die Proteinstoffe, Liebig's Annalen, Band 169, neue Reihe 93, pp. 150–166.

[‡] Otto Nasse, Studien über die Eiweisskörper, Pflüger's Archiv, Band vii, pp. 139–158.

[§] W. Hausmann, Ueber die Vertheilung des Stickstoffes im Eiweissmolekül, Zeitschr. f. physiolog. Chemie, Band xxvii, p. 95.

[||] Hausmann designates as "amide" nitrogen the "loosely combined nitrogen" of Nasse. Monoamino nitrogen is that contained in the amido acids leucin, tyrosin, glutamic acid, etc., and diamino nitrogen that of the bases precipitated by phosphotungstic acid.

TABLE SHOWING THE AMIDE NITROGEN CALCULATED IN PERCENTAGES ON TOTAL NITROGEN.

Reagent	H_2SO_4							HCl (Concentrated)						Hausmann ‡	Nasse	Pröscher §	Pick ‖
Per cent	5	10	10	15	20	40	20	7	7	20	40	25	20				
Period of boiling (hours)	5	10	40	15	20	20	450	7	7	20	40	50	96				
Fibrin (carefully purified)	7.5	7.7		8.0	8.1	9.2		7.9			10.4				10.0		
Hæmoglobin (from dog's blood, twice recrystallized)	4.6	4.8	11.0	5.0		5.6			9.7	10.2	11.2					2.3	
Edestin (crystallized from hemp seed)	9.7	9.2		9.9	9.9	10.5		7.1	7.5	8.5	9.5			8.5	11.2		
Egg albumin (twice recrystallized)	6.5	7.1		7.4	7.7	8.1		21.1									
Zein (from maize) *								10.2									
Casein (prepared by Hammarsten's method)	9.8	10.2		10.9	9.9	10.4		10.0	10.1	10.6	12.0	13.3		13.3	12.5		
Casein (commercial)								6.2						6.3	8.9		
Serum albumin (commercial) *								1.1	1.0		1.9	2.6		1.6	3.8		
Gelatin (commercial)								17.0									
Ovomucoid *																	
Witte's pepton (compare with fibrin)								4.0									
Heteroalbumose (from Witte's pepton)												9.3					6.45
Protoalbumose "																	7.14
Antialbumid †													4.8				
Hemialbumose †													6.0				
Hemipepton †							10.0						6.5				

* These determinations were carried out by Professor Lafayette B. Mendel, and are published by his kind permission.

† The antialbumid, hemialbumose, and hemipepton were prepared from egg albumin by boiling for twenty hours in ten per cent sulphuric acid. According to the views of Hausmann, this treatment should have split off practically all of the amide nitrogen, and these preparations should contain only monoamino and diamino nitrogen. The results show, on the contrary, that by further treatment with acid an additional four to ten per cent of the nitrogen can be separated as ammonia.

‡ The proteids investigated by Hausmann were boiled for five hours in concentrated hydrochloric acid. Loc. cit.

§ Fr. Pröscher, Ein Beitrag zur Erforschung der Constitution des Eiweissmoleküls. Zeitschr. f. physiolog. Chemie, Band xxvii, p. 114.

‖ Pick employed the method of Hausmann. Dr. Ernst P. Pick, Zur Kenntniss der peptischen Spaltungsprodukte des Fibrins. I. Theil, Zeitschr. f. physiolog. Chemie, Band xxviii, p. 219.

percentage figures as constant, and in particular he characterizes the amount of amide nitrogen split off "as a sharply determinable and for each proteid a very characteristic quantity."

To settle this question fully, the writer has performed a series of investigations in which proteids purified with the utmost care were boiled for various periods with hydrochloric and sulphuric acid of various strengths.* The figures giving the results, and set forth in the accompanying table seem to show that the concentration of the acid used, as well as the duration of boiling, exert a decided influence on the distribution of the nitrogen between the different classes of cleavage products. This was shown most clearly when the proteids were treated with sulphuric acid, evidencing the relation between the strength of the reagent and the period of action on the one hand, and on the other the amount of amide nitrogen split off. With hydrochloric acid also results similar in kind were obtained.

In these investigations the amide nitrogen alone has been determined. Yet since they show that the figures obtained are dependent upon the conditions of experiment, it is immediately evident that every increase or diminution in these figures must be accompanied by a corresponding diminution or increase in the amount of diamino or monoamino nitrogen, or, perhaps, in both. The results obtainable for the monoamino and diamino nitrogen must necessarily be subject to considerable variations, as well as those for the amide nitrogen.

* The method of these determinations was essentially the same as that of Hausmann.

ON THE PHOSPHORUS CONTENT OF THE PARANUCLEIN FROM CASEIN.*

By HOLMES C. JACKSON.

WHEN casein is treated with pepsin-hydrochloric acid, there usually results an insoluble substance which has received attention from a number of investigators. Lubavin † presented the first careful study of this product, which had previously been described by Meissner under the name of dyspeptone. He pointed out that the substance contained organic phosphorus to the extent of 4.6 per cent, the latter varying according to the conditions under which the digestive experiments were carried out.

In 1888 Chittenden,‡ while engaged in the study of the digestive products of casein, carried out a series of analyses of the insoluble residue corresponding to Lubavin's dyspeptone. His preparations contained an average content of phosphorus amounting to 2.57 per cent. It was pointed out, however, that in every case the phosphorus found in the ash of the products analyzed was as great as the total phosphorus observed and Chittenden arrived at the conclusion " that instead of being a phosphorized compound, it (dyspeptone) apparently contains no phosphorus whatsoever, other than that combined with calcium." Particularly noticeable in all his preparations is the large percentage of ash, varying from 12.4 per cent to as high as 15.4 per cent. On the other hand, it may be pointed out that there is a rather striking constancy in the quantity of phosphorus found in these preparations, the significance of which will be referred to later. Chittenden was furthermore

unable to reduce materially the amount of ash by any process of purification.

Since these experiments, the study of the so-called dyspeptone has been resumed by various investigators. Thus, Szontagh [*] similarly prepared a dyspeptone which he found to contain organic phosphorus to the extent of 2.96–3.53 per cent — an amount somewhat less than that observed by Lubavin. Again, results of analyses by C. Willdenow [†] show a phosphorus content (3.85 per cent) agreeing more closely with the figures of Lubavin.

Salkowski,[‡] in a later communication, pointed out that the amount of dyspeptone, henceforth called paranuclein in accord with the more recent nomenclature, varies according to the conditions of proteolysis. After a vigorous digestion as little as 6.7 per cent of the original casein remained undissolved as paranuclein, while under less favorable conditions the separation of paranuclein took place to the extent of even 20 per cent. Still more recently Salkowski [§] has observed that where the proteolysis is carried out under extremely favorable conditions, the separation of paranuclein may be wanting entirely. In the former cases, however, the phosphorus content of the paranuclein varied from 2.11 to 2.41 per cent — the higher content coming from products resulting from the more favorable conditions. Salkowski has found the phosphorus just described to exist in organic combination, and has shown that as much as fifteen per cent of the original phosphorus of the casein may be retained in the paranuclein formed from it.

From analyses of paranuclein (pseudonuclein) made by Sebelien [‖] this author finds a phosphorus content varying from 2.5 to 2.76 per cent. He attributes the lack of agreement in

---

[*] Szontagh, Jahresbericht für Thierchemie, 1892, xxii, p. 170.

[†] Willdenow, Inaugural-Dissertation, Bern, 1893 (Drechsel's laboratory).

[‡] Salkowski and Hahn, Archiv für die gesammte Physiologie, 1894, lix, p. 225.

[§] Salkowski, Centralblatt für die medicinische Wissenschaften, 1893, xxxi, p. 385. Cf. also Alexander, Zeitschrift für physiologische Chemie, 1898, xxv, p. 425.

[‖] Sebelien, Zeitschrift für physiologische Chemie, 1895, xx, p. 446.

the phosphorus content of his various preparations to variations in the extent of proteolysis. Similar results regarding the presence of organic phosphorus in the paranuclein and the effect upon its composition due to changed conditions of digestion have since been made in this laboratory by Dr. J. H. M. Knox.* His preparations with an ash content of 2.8 per cent showed a content of phosphorus equal to 2.98 per cent attributable to organic phosphorus. The paranuclein obtained by gastric digestion of casein from goat's milk gave a content of organic phosphorus (2.7 per cent) agreeing closely with that from cow's milk.

A review of the preceding details indicates that most authors have found a considerable content of *organic* phosphorus in this peculiar cleavage product of casein. The results of Chittenden alone stand in contrast to the others. The data of the various investigators are, however, by no means in accord with one another. They vary very widely according to the conditions under which the experiments have been carried out, both with respect to the quantity of paranuclein formed and the composition of the latter. Furthermore, the large ash content revealed by the analyses of previous investigators makes it clear that they were working with preparations which could scarcely lay claim to any considerable degree of purity. The discrepancies noted and the more recent advances made in the field indicated, have rendered it desirable to repeat some of the older experiments, and especially to ascertain the possibility of the formation of paranuclein, entirely devoid of organic phosphorus as it was assumed by Chittenden to have been formed in his experiments.

DIGESTION I.

In this first experiment, an attempt was made to repeat closely the preparation of paranuclein as outlined by Chittenden.† Casein prepared according to the method of Hammarsten, was thoroughly extracted with ether and dried at

* These results have not yet been published.
† Chittenden, loc. cit.

105° C. for analysis. The following results indicate the purity of the product:

	N. per cent.	P. per cent.
Hammarsten	15.65	0.847
Jackson	15.58	0.852

Casein from fifteen quarts of skimmed milk was placed in seven litres of 0.4 per cent hydrochloric acid and warmed at 38° C. To this was added a dialyzed pepsin solution containing 0.75 grm. of commercial scale pepsin (1 : 3000). This mixture was allowed to digest for two days at 38° C., at the end of which time the undissolved residue was filtered off and washed thoroughly with water. The undissolved matter was again placed in three litres of 0.4 per cent hydrochloric acid containing 100 c.c. of a 1 per cent dialyzed pepsin solution, and allowed to digest at 38° C. for four days. Filtered from this digestive mixture, the resulting residue was treated again with a similar pepsin-hydrochloric acid solution and the proteolysis continued for seven days at 38° C. As the quantity of insoluble matter did not appear to decrease, it was filtered from the acid fluid and washed with water until the washings gave only a faint test for chlorides.

It seemed possible that the apparent absence of organic phosphorus in Chittenden's preparations might be due to the action of the alkali which had been used in redissolving and purifying the paranuclein. In order to determine this point, the paranuclein obtained in the above digestion was divided into two portions, each of which was subjected to the treatment outlined below. Furthermore, in order to preclude the possibility of the formation of a large ash content by the process of dialysis and precipitation employed by Chittenden, the former process was omitted in preparation **B**.

Preparation A. — Paranuclein was dissolved in 1 per cent sodium carbonate solution and filtered, thus removing the larger part of the fat left adherent to the residue of digestion. Upon addition of dilute hydrochloric acid to the alkaline filtrate, the paranuclein was thrown down as a heavy flocculent precipitate. The latter was filtered, washed, and again dis-

solved in 1 per cent sodium carbonate solution. This fluid, after exact neutralization with dilute hydrochloric acid, was thymolized and then dialyzed in running water until all traces of chlorides were removed. The resultant neutral solution of paranuclein was concentrated on the water bath to a thick syrup, and while still warm was precipitated with 95 per cent alcohol. Upon standing for some time the precipitate was filtered, extracted thoroughly with alcohol and ether, and dried at 105° C.* It gave the following analysis:

Ash $= 13.33\%$.
Total phosphorus $= 1.65\%$. Phosphorus found in ash $= 1.73\%$.
Phosphorus in substance in organic combination $= 0.0\%$.

Preparation B. — Paranuclein was placed in water and sufficient sodium hydroxide added to hold the substance in solution. By repeated filtration a perfectly clear solution was obtained, and this was precipitated with acetic acid. The resulting precipitate was washed by decantation with distilled water, filtered, and the substance redissolved and reprecipitated twice according to the method just described. After the final precipitation the paranuclein was treated with alcohol and ether, extracted with ether in a Soxhlet apparatus for several days, and finally dried at 105° C. Analysis gave the following results:

Ash $= 1.68\%$.
Total phosphorus $= \begin{cases} 2.75\%. \\ 2.67\%. \end{cases}$ Phosphorus found in ash $= 0.0\%$
Phosphorus in substance in organic combination $= 2.75\%$.

DIGESTION II.

This digestion was carried out in a manner analogous to that already described, with the exception that all treatment of the resultant paranuclein with alkali as well as the subsequent process of dialysis was avoided. The analysis of the product thus prepared without further purification follows:

* This method of procedure agrees quite closely with that described by Chittenden, loc. cit.

Preparation C. —
$$Ash = 2.91\%.$$
Total Phosphorus $= 2.04\%$.   Phosphorus found in ash $= 0.0\%$.
Phosphorus in substance in organic combination $= 2.10\%$.

DIGESTION III.

In order to obtain further light on the influence which the method of preparation might exert upon the analysis and composition of the product, paranuclein was again prepared according to the method outlined in Digestion I. Portions of the same product were treated as follows:

Preparation D. — This preparation corresponds to preparation **A**. It served as a standard for comparison with the products to follow. Analysis gave:

$$Ash = \begin{cases} 11.80\%. \\ 11.92\%. \end{cases}$$
Total phosphorus $= \begin{cases} 2.82\%. \\ 2.90\%. \end{cases}$ Phosphorus found in ash $= 2.47\%$.
Phosphorus in substance in organic combination $= 0.44\%$.

It will be noticed that, as in the case of preparation **A**, the ash content was very high, and in correspondence with the latter nearly all of the phosphorus was recovered in the ash.

Preparation E. — This preparation received treatment similar to preparation **C**, the final digestive product, however, having been simply dissolved in 1 per cent sodium carbonate solution, filtered, precipitated with hydrochloric acid, again filtered, washed, and prepared for analysis.

$$Ash = \begin{cases} 1.54\%. \\ 1.67\%. \end{cases}$$
Total phosphorus $= \begin{cases} 2.40\%. \\ 2.44\%. \end{cases}$ Phosphorus found in ash $= 0.10\%$.
Phosphorus in substance in organic combination $= 2.36\%$.

Preparation F. — This preparation was treated like **B** with the additional feature that it was subjected to dialysis after repeated solution and reprecipitation by means of acid. It differs from preparation **E** only in the fact that it was carefully neutralized before dialysis. Analysis gave the following results:

$$\text{Ash} = \begin{cases} 1.07\%. \\ 1.09\%. \end{cases}$$

$$\text{Total phosphorus} = \begin{cases} 2.97\%. \\ 2.77\%. \end{cases} \quad \text{Phosphorus found in ash} = 0.14\%.$$

Phosphorus in substance in organic combination $= 2.75\%$.

As in the case of **E**, the ash content was relatively small and practically all the phosphorus existed as organic phosphorus, *i.e.*, disappeared on ignition.

The following summary will assist in the comparison of the analyses of the products obtained.

Preparation.	Ash.	Phosphorus			Method used in Preparation.
		In Para-nuclein.	In Ash.	In Ash-free Substance.	
	per cent.	per cent.	per cent.	per cent.	
A	13.33	1.65	1.73	0	Chittenden's — precipitation with alcohol.
B	1.68	2.71	0	2.75	Redissolved in minimal quantity of alkali and reprecipitated with acid.
C	2.91	2.04	0	2.10	No purification after digestion.
D	11.86	2.86	2.47	0.44	Chittenden's.
E	1.60	2.42	0.10	2.36	Similar to **C** — reprecipitated once with acid.
F	1.08	2.87	0.14	2.75	Similar to **B**, subsequent dialysis.
G	0.66	2.35	0	2.36	Preparation **D** dissolved in alkali and reprecipitated with acid.
H	. . .	. . .	3.03	. . .	Preparation **E** treated with a soluble calcium salt.

It will be observed at a glance that the retention of phosphorus in the ash of the preparation, and accordingly the *apparent* absence of organic phosphorus in the substance, is associated with those preparations only which have a high ash content (**A** and **D**). That this condition is not attributable to the dialysis of the product is evidenced by the fact that **A**, **D**, and **F** were each subjected to the process and that the ash content of the preparation is in no way related to this part of the procedure — **F** being one of the purest products obtained, whereas **A** and **D**, dialyzed under similar conditions, showed a very high ash content. The only respect in which the preparations **A** and **D** differed materially from **F**, was in the fact that, whereas the former were precipitated from their

concentrated neutralized solutions by the use of alcohol, **F** (like all the preparations showing low ash content) was first precipitated by means of acid before subsequent treatment with alcohol and ether.

It seemed reasonable to assume that the high content of ash present in preparations **A** and **D** was due to the simultaneous precipitation of relatively insoluble salts, such as calcium salts or more probably to the fact that when the precipitation takes place in a *neutralized solution* of paranuclein, the latter is carried down in combination with an alkali earth such as calcium. Since the paranuclein behaves like an acid substance, it is reasonable to suppose that it may enter into loose combination with calcium, forming compounds analogous to those of casein with calcium and the alkalies. In this event the large amount of calcium present in the preparation would on ignition tend to hold the phosphorus originally present as organic phosphorus in the form of calcium phosphate in the ash. The discrepancy between the results of Chittenden and other observers would thus be attributable to the fact that he precipitated the paranuclein in combination with calcium or an alkali, and owing to the reaction above described, was led to assume the absence of organic phosphorus in the paranuclein.

In order to test still further the validity of this assumption, a portion of the paranuclein with high ash content (preparation **D**) was redissolved in 1 per cent sodium carbonate solution and reprecipitated with hydrochloric acid. After filtration the precipitate was washed with distilled water until the washings gave no test for chlorides.

Preparation G.— An analysis of this substance after treatment with alcohol and ether and drying at 105° C. gave the following results:

Ash = 0.66%.
Total phosphorus = 2.35%.   Phosphorus found in ash = 0.0%.
Phosphorus in substance in organic combination = 2.35%.

It will be seen that the preparation obtained in this way was an extremely pure product. Thus it is quite possible to

transform the paranuclein compound with a high ash content, *i. e.*, paranuclein-calcium (or paranuclein-sodium), into a paranuclein containing only a very small proportion of ash and readily giving off its entire phosphorus on ignition. The reverse process — the formation of a calcium compound of paranuclein and the consequent cleavage of the phosphorus as inorganic phosphorus on ignition — is further demonstrated by the following experiment.

Preparation H.— A portion of preparation **E** was dissolved in calcium hydroxide. After evaporation of the fluid to a small volume, it was treated with absolute alcohol, and the precipitate thus formed removed by filtration. Analysis of this product, dried at 105° C., resulted as follows:

Phosphorus found in the ash = 3.03%.

Summary.

It is shown that the paranuclein obtained by digestion of casein with pepsin-hydrochloric acid always contains a considerable content of phosphorus in organic combination. The results of previous investigators who found that the phosphorus recovered in the ash of their preparations was equivalent to the total phosphorus content of the paranuclein, are attributable to the high ash content of their products. When the latter is avoided, and thus the formation of inorganic phosphate during ignition is precluded, paranuclein invariably yields over 2 per cent of organic phosphorus.

26

BRIEF CONTRIBUTIONS TO PHYSIOLOGICAL CHEMISTRY.[*]

Communicated by LAFAYETTE B. MENDEL.

I. On the occurrence of iodine in corals, by Lafayette B. Mendel.
II. Glycogen formation after inulin feeding, by R. Nakaseko.
III. The influence of acids on the amylolytic action of saliva, by G. A. Hanford.
IV. On the connective tissue in muscle, by J. H. Goodman.

I.

ON THE OCCURRENCE OF IODINE IN CORALS.

By LAFAYETTE B. MENDEL.

BAUMANN's discovery of iodine as a normal constituent of the animal body, and Drechsel's investigations on the iodine compounds of a Mediterranean coral have served to direct attention again to the physiological rôle of this element.[†] Drechsel demonstrated that the horny axial skeleton of Gorgonia cavolinii which he obtained at Naples contains iodine in organic combination. This skeletal substance, termed *gorgonin* by him, yielded on decomposition with baryta water a well crystallized iodo-amido acid, corresponding in composition with a moniodo-amidobutyric acid. Drechsel did not regard the latter as a distinct component of the gorgonin, but assumed it to be a characteristic cleavage product of the complex iodine-containing, keratin-like albuminoid of the coral skeleton. The further peculiar fact that the cœnenchyma of the animal contains practically no iodine led Drechsel to the interesting conclusion that Gorgonia cavolinii has a specific iodine metabolism which is essential to the building up of the framework of the

---

[*] Reprinted from the Amer. Jour. of Physiol., vol. iv.
[†] Cf. Baumann, Zeitschrift für physiologische Chemie, 1896, xxi, p. 319. Drechsel, Zeitschrift für Biologie, 1896, xxxiii, p. 90.

axial skeleton. Hundeshagen * had previously investigated an iodine-containing organic substance which he separated from marine sponges; and later Harnack † isolated from the same source a compound which he has termed *iodospongin*, containing on an average 8.2 per cent of iodine. Other investigators ‡ also have more recently demonstrated the presence of iodine in organic combination in other marine organisms.

Through the kindness of Professor Verrill I have had an opportunity to examine specimens of three species of corals which were collected in the West Indies. These species,§ Gorgonia flabellum, Gorgonia acerosa, and Plexaura flexuosa, resemble Gorgonia cavolinii in many respects. The latter is, however, distinctly a Mediterranean species, while the others have been found in the West Indies only. Gorgonia flabellum grows to a large size; it is flabellate, and throughout finely reticulate. The fronds are sometimes two feet high and nearly as broad. The color varies from an ash to a bright yellow, and is occasionally red. The polyps are everywhere scattered, except where the wing-like processes commence to grow from the surface, and in that case they become lateral. Gorgonia acerosa is the large, purple, pendulous species of the West Indies. When young, the branchlets are erect or nearly so, and the pinnate character is less distinct than in adult specimens. The latter are very large, often five feet high. The axis is black. There are either one or two rows of polyps on the opposite sides of the branchlets. Plexaura flexuosa has a fulvous or purplish color. The branches are terete and without verrucæ, but have a slightly and minutely uneven surface, owing to the fact that the oscules are either situated in a slight depression of the cortex, or have the inferior side a little prominent. The length of the branchlets is often six inches.

* Hundeshagen, Zeitschrift für angewandte Chemie, 1895, p. 473.

† Harnack, Zeitschrift für physiologische Chemie, 1898, xxiv, p. 412.

‡ Eschle, Zeitschrift für physiologische Chemie, 1897, xxiii, p. 30. Gautier, Comptes rendus de l'Académie des Sciences, 1899, cxxviii, p. 1069.

§ See Dana, Wilkes Exploring Expedition, vii, Zoöphytes, pp. 650, 665, 668.

In each of these species iodine was found to be a constituent of the horny axial skeleton. The organic material was fused with pure sodium hydroxide and potassium nitrate, and the fusion products were tested for iodine in the usual way by acidification and extraction with chloroform. All the reagents used were previously ascertained to be absolutely free from iodine. In order to afford a quantitative comparison with the specimens examined by Drechsel, the adherent material was carefully separated from portions of the air-dry axial skeletons, and the latter comminuted and dried for analysis at 110° C. After fusion in a nickel crucible with pure sodium hydroxide, the halogens were precipitated as silver salts and determined by the method which Drechsel [*] employed. In the table of results, the analyses of Gorgonia cavolinii are added for comparison.[†]

SUMMARY OF THE ANALYSES.

Species.	Loss of weight at 110° C.	Iodine.	Chlorine.
	per cent.	per cent.	per cent.
Gorgonia acerosa.	13.38	1.70	3.17
Gorgonia flabellum (average).	10.44	1.15	1.24
Plexaura flexuosa (average).	13.40	0.28	0.86
Gorgonia cavolinii (Drechsel).	11.16	7.79	2.18

While the results presented are considerably smaller than the figures obtained for Gorgonia cavolinii, they compare more closely with the published analyses of certain algæ [‡] (Fucus vesiculosus, Laminaria digitata) and with Harnack's determinations of the iodine content (1.5 per cent) of ordinary sponges.[§] It is suggestive to note that the species of West Indian coral richest in iodine is the one most closely related to the Mediterranean Gorgonia cavolinii.

In his monograph on " The physiological rôle of mineral

---

[*] Drechsel, Zeitschrift für Biologie, 1896, xxxiii, p. 96.
[†] Most of these analyses were made by A. N. Richards, B. A.
[‡] Eschle, loc. cit.
[§] Harnack, loc. cit. p. 414.

nutrients," Loew * has called attention to the possibility of
the presence of bromine in conjunction with the iodine and
chlorine in the Gorgonias. With a view to ascertaining this
point, I have examined very carefully a relatively large quan-
tity (30 grams) of the skeleton substance of Gorgonia acerosa.
The latter was selected because it was the richest in halogens
of all the species examined. The results were entirely nega-
tive; not a trace of bromine was found. Lastly, the skeleton
substance of Gorgonia flabellum was decomposed (in quantities
of 50 to 75 grams) with baryta water. The method pursued
by Drechsel in isolating his compound $C_4H_8NIO_2$ was closely
followed, but without success. Solutions containing organic
iodine compounds were obtained, but no corresponding amido-
acid could be isolated. Whether this result is due to the
relatively small quantities of the material available,† or to a
difference in the way in which the iodine exists in combination
in these species cannot be definitely stated.

The preceding observations afford further justification for
the belief already maintained by Drechsel, that for many
organisms iodine is as essential an element as is chlorine for
others; and that in the absence of iodine the normal nutrition
of the organism may be interfered with. Without some
assumption of this nature, it is difficult to understand why
organisms like the Gorgonias should store up in their horny
axial skeleton an element existing only in traces in sea water,
and apparently not entering into the constitution of the true
growing cœnenchyma of the animal.

* Loew, U. S. Department of Agriculture; Division of Vegetable Physi-
ology and Pathology, 1899, Bulletin No. 18, p. 21, footnote.
† Drechsel obtained only 0.34 gram of the pure amido-acid from 50 grams
of gorgonin.

II.

GLYCOGEN FORMATION AFTER INULIN FEEDING.

By R. NAKASEKO.

INTRODUCTORY.

THE carbohydrate inulin, which readily breaks down to levulose by hydrolysis with acids, has long been suggested as a suitable foodstuff in those forms of diabetes in which levulose can be utilized.* Sandmeyer,† however, found that inulin was very poorly utilized in diabetic dogs, a large part of the ingested carbohydrate being eliminated again unchanged in the fæces. In view of the marked effect which levulose, in common with many other carbohydrates, exerts in increasing the glycogen-content of the liver, it was natural to look for a similar result in the case of inulin-feeding. To test this possibility, Miura ‡ carried out a series of experiments on rabbits in Külz's laboratory several years ago. Precaution was taken to procure pure inulin for feeding, and the glycogen of the liver was estimated by the Brücke-Külz method. The rabbits had all been starved for six days to reduce the store of glycogen in the liver to a minimum ; thereupon the inulin was administered in small doses at hourly or half-hourly intervals, in order to offer the most favorable conditions for inversion and absorption. The total quantity of carbohydrate ingested varied from ten to twenty-five grams per animal, and the rabbits were killed ten or twelve hours after the administration of the last dose. As a basis for comparison Miura took the glycogen determinations made by Külz § on fasting rabbits. Külz found as a maximum for the glycogen-content

* Külz, Beiträge zur Pathologie und Therapie des Diabetes Mellitus, Marburg, 1874.

† Sandmeyer, Zeitschrift für Biologie, 1894, xxxi, p. 32.

‡ Miura, Zeitschrift für Biologie, 1895, xxxii, p. 255. The references to the older literature on inulin-feeding are given in this paper.

§ Külz, Festschrift für C. Ludwig, 1890, p. 69 ; Centralblatt für Physiologie, 1890, iv, p. 788.

of the liver after six days fasting 0.9 per cent of glycogen, the equivalent of 0.329 gram of glycogen, or 0.252 gram per kilo of body weight. In nineteen experiments with inulin-feeding as outlined, the glycogen-content of the liver was increased in the majority of cases; in six animals, however, negative results were obtained. Of the thirty-six experiments by six different investigators on inulin-feeding and glycogen-formation, which we have found recorded in physiological literature, nineteen have given negative results. The experiments of Miura are the most careful and satisfactory of all in point of technique. He concludes his research with the following remarks: "Renewed investigation is demanded to determine whether the levulose found (post-mortem) in the various portions of the intestine owes its formation to the action of one or more of the digestive juices, to the acid of the stomach, or to vegetable enzymes derived from the food ingested. In view of the marked increase in the glycogen-content of the liver which follows levulose feeding, the conclusion is inevitable that ingested inulin is either converted to levulose only in part, or too slowly to permit any storing up of glycogen from the quantities of sugar absorbed. Herein, perhaps, lies the explanation of the inconstancy of the experimental results." *

Regarding the behavior of inulin towards amylolytic enzymes, the experiments of Mr. A. B. Siviter † in this laboratory afford an answer. He found that the ordinary amylolytic enzymes such as the ptyalin of saliva, the amylopsin of the pancreatic extract, vegetable diastase and "Taka" diastase — a very active enzyme preparation obtained from Eurotium oryzæ — are without action on inulin. Dilute hydrochloric acid (0.05–0.2 per cent) at 40° C. transforms inulin to levulose. Hydrochloric acid combined with proteids likewise inverts inulin to levulose, but more slowly than corresponding

---

* Miura, Zeitschrift für Biologie, 1895, xxxii, p. 265.

† See Chittenden, Amer. Jour. Physiol. 1898, ii, p. xvii. Cf. also the recent papers by Richaud, Comptes rendus de la société de biologie, 1900, lii, p. 416. Bieri and Portier, ibid., 1900, lii, p. 423.

strengths of *free* acid.   Organic acids, such as oxalic, lactic, and salicylic, also transform inulin in the same way.

In adding a number of new experiments on inulin-feeding to those already referred to, we have also had in mind the negative, yet inconclusive experiments carried out in this laboratory with the resistant carbohydrate lichenin.*   We have desired especially to supplement Miura's investigation by administering large portions of inulin and allowing the absorption to proceed during longer intervals than has heretofore been the case.   It was hoped to counteract in this way the effects of a possible *slow* conversion and absorption of the carbohydrate.   It is difficult, however, to determine upon any satisfactory time-limit, owing to the danger of a subsequent loss of the newly stored-up glycogen during the protracted hunger period.

EXPERIMENTAL.

In these experiments we have followed Miura's method with few variations.   The data thus afforded are summarized in the table below.   The inulin used was obtained from various sources and was purified with alcohol, when necessary, until it gave no reduction with Fehling's solution after being heated three minutes in a boiling water-bath.†   It was free from nitrogenous matter.   The rabbits were starved from five to seven days.   Inulin was suspended in warm water and administered, partly dissolved, with a stomach sound.   The animals were killed by decapitation; the liver was quickly removed, and the glycogen determined by the Brücke-Külz method.   The stomach and intestinal contents were tested with Fehling's solution and by Seliwanoff's reaction (HCl and resorcin) for levulose or inulin, other sugars which give the reaction, such as saccharose, being assumed to be absent after long fasting.   The observations on these reactions are not recorded here, since they afford no new points of interest.   Other details are included in the table, and two experiments with levulose are added for comparison.

* Cf. Brown, Amer. Jour. Physiol. 1898, i, p. 458.
† As advised by Kiliani.   Cf. Miura, loc. cit., p. 260, note 1.

	Levulose feeding.		Inulin feeding.						
No. of Experiment.	9	8	7	6	5	4	3	2	1
Initial Body-Weight. (grm.)	2450	2075	2120	2200	2575	1475	1925	1975	1650
Final Body-Weight. (grm.)	2450	1640	1820	1920	2275	1260	1600	1750	1875
Duration of Fasting. (Days.)	0	5	5¼	7	5¼	5¼	6	7	7
Amount of Substance Fed at each Dose. (grm.)	4-12	4-8	4-8	2-8	4-8	4	2	2	2
Total Quantity of Carbohydrate Fed. (grm.)	16	32	33	25	23	20	20	20	18
Interval Between First and Last Feeding. (hrs.)	21½	30	48	41	48	26	12	12	10
Interval Between Last Feeding and Death. (hrs.)	15	17	13	5	12	3	12	10¼	8
Weight of the Liver. (grm.)	88	42	38	89	62	33	40	33	86
Total Glycogen Found. (grm.)	4.6488	2.0640	0.2411	0.7555	0.6087	0.4066	0.2049	0.0518	0.0223
Percentage of Glycogen in the Liver. (per cent.)	5.28	4.91	0.64	1.94	0.98	1.23	0.51	0.20	0.06
Glycogen per Kilo of Final Body-Weight. (grm.)	1.896	1.259	0.183	0.390	0.224	0.323	0.129	0.030	0.016

Conclusion.

A study of the figures presented reveals the fact that an increase of glycogen in the liver above the starvation maximum (0.9 per cent, or 0.252 gram per kilo) ascertained by Külz was obtained in only three cases after inulin-feeding (experiments 4, 5 [?], 6). Any connection between the glycogen-content and the length of time during which the feeding continued is not evident from the data obtained. The well known glycogen-forming property of levulose is again demonstrated in the last experiments and stands in striking contrast to the practically negative results with comparable quantities of inulin. Furthermore, we recall the statement of Külz * — an authority on glycogen-formation — that occasionally there are to be found in the liver of the rabbit quantities of glycogen presumably too large to disappear to the extent recorded in the usual experiments, even after six days' fasting. In addition to this the stomach of the fasting rabbit normally always contains particles of food residue which may gradually offer available carbohydrate.

In view of all these facts, which apply equally to Miura's experiments and to our own, the glycogen-forming properties of inulin, in the case of the rabbit at least, must still be regarded as uncertain or minimal.†

III.

THE INFLUENCE OF ACIDS ON THE AMYLOLYTIC ACTION OF SALIVA.

By G. A. HANFORD.

Introductory.

A series of experiments on the influence of acids and alkalies upon the amylolytic action of the saliva has recently been

* Külz, Centralblatt für Physiologie, 1890, iv, p. 789.
† Cf. also Richaud, Comptes rendus de la société de biologie, 1900, lii, p. 416.

undertaken by F. Kübel * in Professor Grützner's laboratory. The results obtained are largely confirmatory of investigations carried out long ago in this laboratory by Chittenden and Smith.† As Kübel was apparently not familiar with this work, and inasmuch as other recent writers ‡ have overlooked some of the results long established, it seemed desirable to review the older observations here very briefly and to add some experiments of our own which may serve to explain and extend the work of Kübel.

From careful quantitative experiments, in which the extent of amylolytic action was determined by a direct estimation of the sugar formed, Chittenden and Smith concluded as follows :

" The most favorable condition for the diastatic action of ptyalin, under most circumstances, appears to be a neutral condition of the fluid together with the presence of more or less proteid matter. The addition of very small amounts of hydrochloric acid, however, to *dilute* solutions of saliva, giving thereby a *small percentage* of acid-proteids, appears to still further increase diastatic action. Under *such conditions* a minute trace of free acid appears to still further increase the action.

" 0.003 per cent free hydrochloric acid almost completely stops the amylolytic action of ptyalin. The larger the amount of saturated proteids, the more pronounced becomes the retarding action of free acids.

" The retarding effects of smaller percentages of free acid are not due wholly to destruction of the ferment. Pronounced destruction takes place with 0.005–0.01 per cent free hydrochloric acid.

" Proteid matter, in influencing the diastatic activity of salivary ptyalin acts not only by combining with acids and

---

* Kübel, Archiv für die gesammte Physiologie, 1899, lxxvi, p. 276.

† Chittenden and Smith, Studies from the Laboratory of physiological Chemistry, Yale University, 1885, i, p. 1. Also transactions of the Connecticut Academy, 1885, vi, p. 343: Jahresbericht für Thierchemie, 1885, xv, p. 256.

‡ *E. g.*, Austin, Boston Medical and Surgical Journal, 1899, cxl, p. 325.

alkalies, but apparently also by direct stimulation of the ferment." *

Kübel employed a method introduced by Paschutin for the estimation of relative amylolytic activity: at the end of each trial digestion equal quantities of potassium hydroxide solution were added to the digestion mixtures, and the whole was then plunged into a boiling water-bath for a definite interval. In order to estimate more accurately the extent of digestive action from the intensity of the yellow-brown color brought about by the reaction of the caustic alkali with the soluble products of amylolysis (Moore's reaction), Kübel devised a colorimetric procedure suggested by that of Grützner for pepsin determinations. Solutions of potassium bichromate in various definite dilutions were employed for the color comparisons. Although a method of this character is less exact than direct quantitative determinations of the sugar formed, it gives quite comparable results and makes it possible to carry out a very large number of estimations at one time. From such experiments Kübel concluded that for a two per cent starch paste, acids of $\frac{1}{500}$ n or weaker strengths considerably facilitate the amylolytic action of human mixed saliva.†
Greater concentration of acid tends to inhibit the amylolytic power, the degree of inhibition varying, for equimolecular strengths, with the acids used.

A comparison of Kübel's work with the earlier investigations from this laboratory makes it evident that his conclusions have for the most part been anticipated. Thus Chittenden and Smith clearly demonstrated that small percentages of acid-proteids tend to increase amylolytic action, and that a minute trace of *free* acid appears to facilitate the action still further. Kübel's results, while they are comparable with one another, will not permit any general conclusion such as he has drawn regarding the *exact percentages* of acids or alkalies which stimulate or inhibit amylolytic activity; for the work of previous

* Chittenden and Smith, Studies from the Laboratory of physiological Chemistry, Yale University, 1885, i, p. 83.

† Kübel, Archiv für die gesammte Physiologie, 1899, lxxvi, p. 303.

investigators has emphasized the important influence which the presence of proteids may exercise — a fact apparently overlooked by Kübel. Proteid matter tends to prevent the destructive or inhibitory influence of acids by combining with them; in comparing the rate of digestive action under different conditions, it is obviously necessary to take into consideration the amount of proteid present. Thus the dilution of the saliva, or the differences in the concentration of the secretion collected at different times or from different individuals may bring about digestive variations when all the other conditions are constant, owing to the varying quantities of proteid thus introduced. Further progress was made when it was demonstrated by Chittenden and Smith that *free* acid, *i. e.*, acid which reacts with tropæolin 00, readily destroys the enzyme of the saliva. The difference between free and combined acid in their relative destructive or inhibitory power appears to have been overlooked by Kübel. It will be shown in the experiments to follow that digestion never proceeds at all in the presence of more than the merest traces of *free* hydrochloric acid; and it would seem probable from our repetition of Kübel's experiments that in those instances in which he observed complete inhibition of digestive action, *free* acid was usually present. The conception of *free* acid as an inhibitory agent towards ptyalin carries with it a definite idea; it is independent of any consideration of the accompanying conditions, such as amount of saliva used, concentration of saliva or strength of starch paste. Whenever the digestive mixture gives a reaction for *free* acid, no amylolysis is to be expected. On the other hand, to speak of a definite total acidity of the fluid as influencing amylolysis in a definite way is scarcely permissible without defining carefully the conditions of the reaction, in particular the amount of proteid present. Even the different proteids vary widely in their combining power with acids.

Experimental.

In the part following we shall attempt to demonstrate some of the points just discussed, by extracts from the protocols of our experiments. Our method has been similar to that of Kübel. Starch paste made from pure, neutral, arrowroot starch; acid of known strength; and lastly saliva variously diluted, have been mixed together, and the extent of amylolysis at different temperatures determined after various intervals (usually ten minutes). Kübel's colorimetric method was followed in estimating the relative rate of digestion. The presence of *free* hydrochloric acid was tested for by the delicate reaction with dimethyl amidoazobenzol first introduced by Töpfer.*

First Series. — The experiments of this series illustrate how the individual conditions may influence the limits of amylolytic action independently of the quantitative relations of the reagents which enter into the digestive mixture. They are intended to contrast with Experiment 19 of Kübel. As in his experiment, so here, a four per cent neutral starch paste was used; mixed human saliva was diluted with one part of water, filtered, and finally diluted with three parts of glycerine. Different lots of saliva were employed. Under such conditions Kübel found — with his own saliva and wheat-starch paste — that in a mixture containing five c.c. of starch paste, 0.3 c.c. of saliva, and five c.c. of acid of known strength, the digestion at room temperature and during a period of ten minutes was facilitated by hydrochloric acid of $\frac{1}{5000}$ to $\frac{1}{4000}$ n resultant strength, and entirely checked by $\frac{1}{200}$ n or stronger hydrochloric acid. In the typical protocols tabulated (Tables A, B) it will be seen how the limits of digestive action may vary with saliva from different sources, etc. It will also be noted that *digestion has not proceeded* in those experiments in which *free* hydrochloric acid was detected.

The favorable action of very small quantities of acids (*com-*

---

* Töpfer, Zeitschrift für physiologische Chemie, 1894, xix, p. 104.

TABLE A.

Temperature, 17° C. Period of Digestion, 10 minutes.

Starch Paste.	Diluted Saliva.	HCl Added.	Resultant Acidity as HCl.	Experiment I.		Experiment II.		Experiment III.	
				Reaction for *free* HCl.	Results.	Reaction for *free* HCl.	Results.	Reaction for *free* HCl.	Results.
c.c.	c.c.	c.c.	per cent.						
5	0.8	$5H_2O$	0.0000	−	Digestion.	−	Digestion.	−	Digestion.
5	0.8	$5\frac{n}{1000}$	0.0036	−	No digestion.	−	Digestion.	−	No digestion.
5	0.8	$5\frac{n}{500}$	0.0072	+	No digestion.	−	No digestion.	−	No digestion.
5	0.8	$5\frac{n}{200}$	0.0182	+	No digestion.	+	No digestion.	+	No digestion.

TABLE B.

In these experiments each test tube contained 5 c.c. starch paste, 0.3 c.c. dilute saliva, 5 c.c. HCl of the strength indicated. Temperature, 17° C. Period of digestion, 10 minutes.

No. of Tube.	HCl added.	Total Acidity as HCl.	Reaction for *free* HCl.	Results.*		
				Experiment I.	Experiment II.	Experiment III.
	c.c.	Per cent.				
1	5 H_2O	0.0000	—	Digestion, 5.	Digestion, 5.	Digestion, $2\frac{1}{4}$.
2	$5\frac{n}{1000}$	0.0036	+	No digestion.	No digestion.	No digestion.
3	$5\frac{n}{2000}$	0.0018	—	Digestion, 5.	Digestion, 2.	Digestion, $2\frac{1}{4}$.

bined acid in every case) is demonstrated, in agreement with previous observers, by the following protocol : —

TABLE C.

Period of digestion, 10 minutes. Temperature, 37° C.

No. of Tube.	Diluted Saliva Added.	HCl Added.	Total Acidity as HCl.	Reaction for *free* HCl.	Result.
	c.c.	c.c.	Per cent.		
1	0.3	5 H_2O	0.0000	—	Digestion, 2.
2	0.3	$5\frac{n}{500}$	0.0043	—	No digestion.
3	0.3	$5\frac{n}{1600}$	0.0021	—	Digestion, 5.
4	0.3	$5\frac{n}{3200}$	0.0010	—	Digestion, 5.
5	0.3	$5\frac{n}{6400}$	0.0005	—	Digestion, 3.

TABLE C (*continued*).

Period of digestion, 10 minutes. Temperature, 17° C.

No. of Tube.	Diluted Saliva Added.	HCl Added.	Total Acidity as HCl.	Reaction for *free* HCl.	Results.		
					Exp. I.	Exp. II.	Exp. III.
	c.c.	c.c.	per cent.				
1	0.3	5H_2O	0.0000	—	Digestion, 4.	Digestion, 3.	Digestion, 4.
2	0.3	$5\frac{n}{1000}$	0.0036	+	No digestion.	No digestion.	No digestion.
3	0.3	$5\frac{n}{1500}$	0.0024	—	Digestion, 10.	Digestion, 2.	Digestion, 2.
4	0.3	$5\frac{n}{2000}$	0.0018	—	Digestion, 5.	Digestion, 4.	Digestion, 5.

* The figures refer to the artificial color scale indicating relative extent of amylolysis.

Second Series. — The following experiments are selected to illustrate how an increase in the amount of neutral saliva added may modify the influence of a definite proportion of acid, presumably owing to a combination of its proteid with the latter. The relatively weaker inhibitory action of combined acid is thus demonstrated.

TABLE D.

Period of digestion, 10 minutes.

No. of Tube.	Diluted Saliva Added.	HCl Added.	Total Acidity as HCl.	Results.	
				Exp. I. Temp. 40° C.	Exp. II. Temp. 17° C.
	c.c.	c.c.	per cent.		
1	3.3	$2H_2O$	0.0000	Digestion, 5.	Digestion, 3.
2	3.3	$2\frac{n}{1000}$	0.0036	No digestion.	No digestion.
3	3.3	$2\frac{n}{2000}$	0.0018	Digestion, 12.	Digestion, 5.

In Experiment I, 3.3 c.c. saliva were added to Tube 2, whereupon the mixture was digested, and gave a color comparable to No. 4 of the color scale. In Experiment II, it required three successive additions of 3.3 saliva to bring about digestion.

TABLE E.

Period of digestion, 10 minutes. Temperature, 17° C.

No. of Tube.	Diluted Saliva Added.	HCl Added.	Total Acidity as HCl.	Reaction for *free* HCl	Result.
	c.c.	c.c.	per cent.		
1	0.3	$5H_2O$	0.0000	—	Digestion, 5.
2	0.3	$5\frac{n}{1000}$	0.0036	+	No digestion.
3	0.3	$5\frac{n}{1500}$	0.0024	?	No digestion.
4	0.3	$5\frac{n}{2000}$	0.0018	—	Digestion, 10.

At the end of ten minutes 0.3 c.c. saliva was again added to Tubes 2 and 3. Tube 2 still gave an acid reaction, while Tube 3 digested at once, and at the end of ten minutes gave with potassium hydroxide a color reaction slightly weaker than that obtained with Tube 1.

In the following protocols the effect of the reverse process, namely, diminishing the amount of neutral saliva added when

27

the other conditions are unchanged, is illustrated. (See Table F, page 419.)

Third Series. — The influence of increased quantities of neutral starch paste in modifying the inhibitory effect of acid is shown below.

TABLE G.

Period of digestion, 10 minutes. Temperature, 37° C.

No. of Tube.	Starch Paste Added.	Water Added.	HCl Added.	Total Acidity as HCl.	Diluted Saliva.	Result.
	c.c.	c.c.	c.c.	per cent.	c.c.	
1	1	4	$5\,\frac{n}{1000}$	0.0036	0.3	No digestion.
2	2	3	$5\,\frac{n}{1000}$	0.0036	0.3	No digestion.
3	3	2	$5\,\frac{n}{1000}$	0.0036	0.3	Digestion 2.
4	4	1	$5\,\frac{n}{1000}$	0.0036	0.3	Digestion 4.
5	5	0	$5\,\frac{n}{1000}$	0.0036	0.3	Digestion 5.

In Table H the effect of acid of varying strength upon the saliva is shown. It was first ascertained that five c.c. of saliva carefully neutralized to litmus required exactly five c.c. $\frac{n}{100}$ n hydrochloric acid to give the faintest reaction with dimethyl amidoazobenzol. Equal volumes of saliva were then mixed with varying quantities of hydrochloric acid, the total volume in each case being kept the same. At the end of the intervals indicated, the mixtures were carefully neutralized to litmus, and their digestive power tested with starch paste. The results of two experiments are given in Table H.

TABLE H.

No. of Tube.	Diluted Saliva.	Water Added	$\frac{N}{100}$ HCl Added.	Total Acidity as HCl.	Results.	
					Exp. I. 30 minutes at 17° C.	Exp. II. 1½ hours at 40° C.
	c.c.	c.c.	c.c.	per cent.		
1	5	10	10	0.0146	No digestion.	No digestion.
2	5	12	8	0.0116	No digestion.	No digestion.
3	5	13	7	0.0102	Digestion.	No digestion.
4	5	14	6	0.0081	Digestion.	No digestion.

TABLE F.

Period of digestion, 10 minutes. Temperature, 37° C.

No. of Tube.	HCl Added.	Total Acidity as HCl.	Experiment I.				Experiment II.			
	c.c.	per cent.	Diluted Saliva. c.c.	Result.	Diluted Saliva. c.c.	Result.	Diluted Saliva. c.c.	Result.	Diluted Saliva. c.c.	Result.
1	5 H_2O	0.0000	1.6	Digestion, 3.	3.3	Digestion, 4.	0.3	Digestion, 5.	0.6	Digestion, 10.
2	$5\frac{n}{1000}$	0.0035	1.6	No digestion.	3.3	Digestion, 3.	0.3	No digestion.	0.6	Digestion, 1.
3	$5\frac{n}{1150}$	0.0029	1.6	No digestion.	3.3	Digestion, 4.	0.3	Digestion, 3.	0.6	Digestion, 3.
4	$5\frac{n}{1500}$	0.0024	1.6	Digestion, 5.	3.3	Digestion, 4.	—	—	—	—

From these data it will be seen, as has already been pointed out by Chittenden and Smith, that *free* hydrochloric acid, especially when it reacts for any length of time, is destructive to the enzyme of the saliva, even with extreme dilution of the acid.

With reference to the retarding effect of alkalies upon salivary digestion as ascertained by Kübel, we recall that Chittenden and Smith * also pointed out the inhibitory reaction in the case of sodium carbonate. But in this instance they likewise emphasize the importance of considering the dilution of the saliva and consequent changes in the amount of proteid present, before any definite statement can be arrived at for definite strengths of alkali.

In discussing the fate of the saliva in the stomach during digestion, it will hereafter be necessary to take into consideration the investigation of Cannon † on the absence of movement in the fundus, if the observations on the cat and dog be applicable to other animals and man also. The food is not readily mixed with the gastric juice in this portion of the organ, and consequently an acid reaction does not develop for some time. Salivary digestion may thus presumably proceed for some time in this region without marked retardation.

Summary.

The chief object of this note has been to point out that it is impossible to designate any percentage of acid or alkali which inhibits salivary digestion in a definite degree. The character of the action is dependent also upon the absolute amount of saliva and the attendant variation in the quantity of proteid matter present. Whenever *free* hydrochloric acid is present, inhibition — more or less complete — is certain to result.

* Chittenden and Smith, Studies, etc., p. 33.

† Cannon, Amer. Jour. Physiol., 1898, i. p. 379.

IV.

ON THE CONNECTIVE TISSUE IN MUSCLE.

By J. H. GOODMAN.

IN a paper from the hygienic institute in Würzburg, E. Schepilewsky * has quite recently described a new method for the determination of connective tissue in muscle. Strips of meat are gently rubbed in a mortar with water, the latter being repeatedly renewed. It is possible to remove the bulk of the true muscular tissue in this mechanical way, and to retain the meshwork of connective tissue. The water is next poured through a fine sieve which holds the detached pieces of connective tissue, but allows the muscle elements to pass through. According to Schepilewsky, practically all of the connective tissue can thus be retained, while only small quantities of muscle fibres themselves are held back in the meshwork of tissue. The latter is now rubbed up with five per cent sodium hydroxide solution and allowed to stand in contact with the alkali for some time. The true muscle elements are found entirely dissolved at the end of fifteen hours; the connective tissue swells up and becomes transparent, showing the elastic fibres in clear outline. The undissolved mass is thereupon filtered through a perforated porcelain plate covered with cotton-wool and is washed well with water; the entire residue, including cotton, is next boiled gently for five or ten minutes with a small volume of half per cent sodium hydroxide solution. The collagen passes into solution in the hot alkali, leaving the elastic fibres undissolved. By determining the nitrogen in a portion of the filtered solution, the amount of collagen dissolved can readily be estimated. In discussing the details of the method, Schepilewsky writes as follows, regarding the action of the strong alkali: " The lye dissolves the proteids, at the same time saponifying the fats and extracting from the connective tissue the greater part of the *mucin ;* the latter is

* Schepilewsky, Archiv für Hygiene, 1899, xxxiv, p. 348.

readily detected by adding an excess of acetic acid to the filtered alkaline fluid. The proteids present in the latter are redissolved by this procedure, while the *mucin* is precipitated in flocks." Again, in reference to the final alkaline fluid obtained after the treatment with the more dilute alkali, it is stated : " There may still be present a small quantity of *mucin,* which is readily removed by adding acetic acid in excess and filtering off the precipitate formed after some time ; fatty acids are also retained on the filter." [*]

Although we have been unable to find any reference to the occurrence of mucin in muscles, it did not seem improbable that this compound proteid might be present; for mucin is a characteristic component of white fibrous connective tissue, and apparently also of bone.[†] We have therefore separated the connective tissue from muscle and isolated the so-called mucin described by Schepilewsky. Three different samples of muscular tissue were examined, two of which were lean beef, the third being rabbit's muscle. In each instance about five hundred grams of the meat were chopped up, rubbed in a mortar repeatedly with water and strained, until practically all of the muscle fibres were eliminated. The connective tissue residue was then placed in five per cent sodium hydroxide solution for about fifteen hours. The resulting extract was strained off and filtered ; the filtrate was acidified with acetic acid and the flocculent precipitate obtained was washed thoroughly with water by decantation, with alcohol and ether, and dried at 105° C. This was preparation A, which might be made up in part or entirely of mucin, as the latter is readily soluble in alkalies, but insoluble in excess of acid. The residue undissolved after treatment with the caustic alkali was treated with half per cent sodium hydroxide solution, in which the collagenous tissue dissolved together with some saponified fats. The solution was filtered and acidified with an excess of acetic acid, which threw down a precipitate of proteid and fatty acids. This precipitate would not dissolve completely in a

[*] Schepilewsky, loc. cit., pp. 357–358.
[†] Gies, Amer. Jour. Physiol., 1900, iii, p. vii.

very large excess of acid; on treatment with boiling alcohol, nearly all of it went into solution as fatty acid, leaving only a trace of proteid behind. Portions of fatty acid obtained in this way melted above 62° C.

In order to determine the identity of preparation A as mucin, portions of about 1.5 grams were heated for fourteen hours with two per cent hydrochloric acid. It was hoped to bring about a cleavage of the glycoproteid in this way, if mucin were present.* The solution was nearly neutralized (never becoming alkaline), and was concentrated to a small volume. The presence of a soluble carbohydrate was tested for with Fehling's solution. No reduction was ever observed, and *no carbohydrate group could be detected in any preparation of the so-called mucin.* Furthermore, the mucins are all characterized by a relatively low content of nitrogen in contrast with simple proteids. Thus tendon mucin contains less than 12 per cent of N, while snail mucin and submaxillary mucin contain 13.6 and 12.3 per cent respectively.† Analyses of the preparations from muscle gave far higher results, as will be seen in the summary below. The nitrogen determinations were made in duplicate by the Kjeldahl-Gunning method. The

ANALYSES OF PREPARATION A.

Source.	Ash.	Nitrogen.	Carbohydrate.
	Per cent.	Per cent.	
1. Beef	0.66	16.07	None.
2. "	0.84	16.22	None.
3. Rabbit	1.20	16.02	None.

figures given are calculated for the ash-free substance. Since the nucleoproteids resemble the true mucins in their solubilities, phosphorus determinations were made by Hammarsten's method, in order to ascertain whether the material under investigation belonged to the former class. Only traces of phosphorus—less than 0.01 per cent—were found, and these were evidently due to adherent phosphate also detected in the ash.

* Chittenden and Gies, Journal of Experimental Medicine, 1896, i, p. 186.
† Halliburton, Schaefer's Textbook of Physiology, 1898, i, p. 62.

From the preceding considerations it is evident that the material assumed by Schepilewsky to be mucin is neither a glycoproteid nor a nucleoproteid. Its composition and solubilities recall the *stroma substance* of J. von Holmgren.[*] This investigator, repeating Danilewsky's experiments,[†] treated the insoluble muscle stroma remaining after complete extraction of muscle with water and ammonium chloride solution, with dilute alkali. He obtained in this way from horse and rabbit muscle a proteid precipitable by acids and yielding neither xanthin bases nor reducing substances. Von Holmgren's stroma proteid contained from 15.84 to 16.66 per cent of nitrogen.

In speaking of the final filtrate — the gelatin solution obtained by dissolving the connective tissue in hot alkali — Schepilewsky says: "It gives no coloration with Millon's reagent, if the proteids have actually been removed."[‡] It has been taught quite universally that pure gelatin does not give Millon's reaction; the latter, when obtained with gelatin solutions, is attributed to contaminating proteids.[§] But the investigations of Van Name[||] in this laboratory have demonstrated that perfectly pure gelatin, prepared from connective tissue, still gives a red coloration when warmed with Millon's reagent; and this observation has received confirmation in the recent work of C. Th. Mörner.[¶] In order to test the matter still further we have neutralized and concentrated the gelatin-containing filtrates obtained by Schepilewsky's method, and have precipitated the albuminoid material with alcohol. The precipitate of gelatin (or gelatoses) gave the characteristic red coloration with Millon's reagent.

[*] Von Holmgren, Jahresbericht für Thierchemie, 1893, xxiii, p. 360.

[†] Danilewsky, Zeitschrift für physiologische Chemie, 1882, vii, p. 124.

[‡] Schepilewsky, loc. cit., p. 358.

[§] *E. g.*, Neumeister, Lehrbuch der physiologischen Chemie, 1897, p. 63. Salkowski, Practicum der physiologischen und pathologischen Chemie, 1900, p. 199.

[||] Van Name, Journal of Experimental Medicine, 1897, ii, p. 117.

[¶] C. Th. Mörner, Zeitschrift für physiologische Chemie, 1899, xxviii, p. 484.